National Institute of Dental Research

ANNUAL REPORT

Fiscal Year 1984

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Services
National Institutes of Health
National Institute of Dental Research

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EPIDEMIOLOGY AND ORAL DISEASES
PREVENTION PROGRAM

ANNUAL REPORT OF THE EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH

As of January 1984, the newly organized Epidemiology and Oral Disease Prevention Program began operation, staffed largely with personnel from the former National Caries Program. The E&ODP Program is charged with the conduct of descriptive and analytical epidemiologic studies of all oral diseases as well as the initiation of controlled clinical trials of potential disease-preventing agents and regimens.

During the few months of its existence, E&ODP staff have given highest priority to completing plans for a nationwide survey of the prevalence of oral disease in adults and the aged. This study will begin in February 1985, and will generate the most comprehensive set of data yet assembled on the dental conditions of U.S. adults. In addition, plans are well developed to repeat the national survey of the prevalence of caries in school children, completed in 1980. It is essential to continue to monitor caries levels in children to determine whether the downward trend in prevalence, observed during the 1970s, will continue.

Although many believe that the decline in dental caries is attributable to widespread use of fluorides, this hypothesis remains unproven, and requires further study. To that end, discussions have begun regarding a collaborative study of caries trends in West Germany, where fluoride programs have never been implemented.

Clinical trials are continuing on the effectiveness of a controlled release system for intraoral delivery of fluoride, on the effects of prenatal exposure to systemic fluorides and on the possibility that dental plaque can be controlled by the use of the antimicrobial, octenidine, as a mouthrinse.

New epidemiologic studies, designed during the year for implementation in FY 1985, include investigation of the prevalence of marginal loss of alveolar bone in adolescents and its possible value in predicting susceptibility to adult periodontitis. Further studies of the prevalence of enamel fluorosis are also planned, to continue to monitor levels of ingestion of fluorides from all sources. Thus far, data which have been collected suggest that, contrary to the concern of some, the prevalence of fluorosis has not increased in recent decades.

During the year, Dr. Jerry Niswander joined the Program staff as Chief, Epidemiology Branch, and is also serving as Acting Chief of the Field Studies Section. Ms. Mary Wolfe and Dr. Edith Morrison joined that Section as Epidemiologists.

The Associate Director was elected to the Executive Board of the European Organization for Caries Research and was the recipient of the PHS Superior Service Award. Dr. Herschel Horowitz, Chief, Clinical Trials Section, received the H. Trendley Dean Award in epidemiology from the International Association for Dental Research.

Annual Report of the Epidemiology Branch
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Epidemiology Branch applies epidemiologic and biometric methods to the study of distribution, cause, prevention, diagnosis and treatment of oral diseases. On abolishment of the National Caries Program, the Epidemiology Branch was created by combining the Biometry Section of the former Caries Prevention and Research Branch with the Microbial Systematics Section of the Intramural Programs. A third component, the Field Studies Section, was created de novo primarily with personnel from several areas of the NCP.

Under this new organization, the Biometry Section will continue to function in much the same manner as previously with the same staff conducting independent research as well as providing data processing and analysis services for the Branch and other programs of the Institute. Likewise, the Microbial Systematics Section continues much as before the reorganization. However, the expertise in microbiology and the capability for handling large files of microbiological data provide an important new resource for epidemiologic studies, particularly for studies of periodontal disease. The new Field Studies Section provides the data collecting resource but will draw heavily upon the other two sections for planning and design of its studies as well as data analysis.

The broad mandate of this new Branch offers many opportunities for new research activities which are presently being developed. Although dental caries will continue to be an area of concern, considerable emphasis is being placed on other oral conditions. The first priority is the development of an active program in periodontal disease. Several new studies have been initiated or are presently in the planning stage.

Annual Report of the Biometry Section
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Biometry Section has collaborated with members of other sections on two major clinical trials several related human and animal immunity studies, S. mutans transmission and caries prevalence studies, and animal studies of bacteria, anti-plaque agents and fluorides. It has also consulted on the design and analysis for three contracts and processed data from six clinical trial contractors.

Staff of the Biometry Section have also participated in the planning of four workshops/conferences on a broad range of topics: nutrition and caries; periodontal clinical trials; epidemiology principles; and caries prevalence, all of which will take place in the coming years. Two papers from the Caries Clinical Trial Workshop of May, 1983, "Stratification Methods in Caries Clinical Trials" and "Use and Misuse of Computer Programs in the Design and Analysis of Caries Clinical Trials," were published this year.

A major task now underway is the processing and tabulation of a two-part survey of oral health of Hispanics, conducted by the National Center for Health Statistics (NCHS). The first part, consisting of approximately 7000 comprehensive dental exams, has been furnished and returned to NCHS. The second part will be conducted until January, 1985. We are expecting to do a brief analysis of caries prevalence on the first and second parts when the survey weights have been added to the raw data tapes.

In conjunction with a clinical trial contract with the University of Michigan on diet and dental caries, a project is underway to analyze the relationship of certain microorganisms in saliva to caries levels in teenagers. The baseline data collected in Fall, 1982 indicated about a one and one-half surface difference between children with high and low levels of S. mutans in their saliva. Saliva from the second exam conducted in Spring, 1984 is being analyzed now and these positive relationships between caries and S. mutans appear to be quite strong again.

Besides consulting with the various laboratory and clinical investigators, we have been working on use of the growth curve model for analysis of microbiological changes in plaque collected over a period of time. This statistical model seems to describe the bacterial activity in a study of natural transmission of S. mutans in rats on various sucrose diets very well.

Using the data from the children's caries prevalence survey of 1979-80, tooth eruption patterns by age, sex and race for U.S. children were determined using probit analysis. It appears that white children may be erupting their teeth earlier than formerly.

Differences in eruption times by race and sex were found, with teeth erupting earlier in females than males and in blacks earlier than whites. The largest difference in age of eruption occurred in upper and lower cuspids, which erupted approximately one and a quarter years earlier in black females than in white males.

The caries prevalence survey data were also used to examine the DMFS prevalence differences between white children and those of other races. Overall, black children still seem to have lower caries prevalence, but differences are quite small. However, blacks and other non-white children in fluoridated cities average about one less DMFS surface than whites. In rural areas and non-fluoridated areas, there are little or no differences between the races.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00292-05 EODP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Analysis of the National Dental Caries Prevalence Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

BRUNELLE, J.A.	CHIEF, BIOMETRY SECTION	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.50	.30	Technical .20

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

A nationwide survey to assess the prevalence of dental caries throughout the United States was designed and implemented during the 1979-80 school year. A probability sample of school-aged children in grades kindergarten through twelve was selected for examination for dental caries, gingivitis, and need for restorative treatment. Estimates of disease level and treatment need were made for the continental U.S. and each of seven geographic regions by age, race and sex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00324-04

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Methods of Analyzing Fluorosis Evaluations Made by Dean's Scoring System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

KINGMAN, ALBERT STATISTICIAN EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

EPIDEMIOLOGY BRANCH

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.20	.20	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Two methods for analyzing fluorosis data based on Dean's classification system are presented and illustrated. The data are derived from examination of four groups of children, having 1, 2, 3 and 4 times optimal fluoride concentrations in their water systems. The first method, ridit analysis, is based on the rank ordering of the data, and provides an estimate of the probability that a randomly selected subject from one group has a more severe level of fluorosis than a randomly selected subject from another group. The second method used to compare groups is based on the number of fluorosed teeth of the subject. Each method identified the same three statistically distinguishable levels of fluorosis relative to fluoride concentration: the optimal group had the lowest level, the 2 and 3 times optimal groups a higher level, and the 4 times optimal group the highest level. The correlation found between Dean's Index and the number of fluorosed teeth for the same subject was 0.77.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00353-03

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A More Efficient Use of Initial DMFS in Predicting Caries Incidence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

KINGMAN, ALBERT STATISTICIAN EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
EPIDEMIOLOGY

SECTION
BIOMETRY

INSTITUTE AND LOCATION
NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.01	.01	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prediction models for the development of dental caries have been investigated by many researchers. To date, the variables shown most often to be correlated with the development of dental caries are the subject's DMFS prevalence and the number of permanent surfaces that exist in the dentition. In a previous study, Kingman has shown that the incorporation of the various surface susceptibility levels for caries into the subjects's caries prevalence assessment can improve models used for caries prediction.

The current investigation focuses on differences in surface susceptibility levels to caries attack and also the separate counts of Dm F and M surfaces diagnosed for the subject. A weighted caries experience is derived using these factors and tested for its predictive power, using recently conducted clinical trial data sets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00354-03 EODP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods of Analyzing Microbiological Changes in Plaque

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

LI, SHOU-HUA STATISTICIAN (Vis.) EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

EPIDEMIOLOGY BRANCH

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS .40	PROFESSIONAL .40	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three statistical methods of analyzing repeated measurements on microbiological changes in plaque were investigated. The simplest method to use is the univariate analysis of variance. This approach has an exact F distribution when hypotheses are tested under restrictive assumptions of normality and equal variances and covariances. An alternative approach called profile analysis is based on the multivariate analysis of variance technique. In the profile analysis, the readings on each subject at t times are regarded as a t-dimensional vector. A third approach is based on the polynomial growth curve model, i.e., a separate polynomial growth model is fit for each group of data.

Data from an eight-week study on the natural transmission of S. mutans in rats on five different dietary concentrations of sucrose was used to illustrate these methods. Statistical analysis indicated that the assumption of equal variance and covariance in the univariate analysis of variance was violated. It was shown that different sucrose concentrations in the diets produced significantly different S. mutans growth profiles. Each sucrose group over time was summarized by a single polynomial equation of time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00358-02

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stratification Methods in Caries Clinical Trials

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert	Statistician	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
EPIDEMIOLOGY BRANCH

SECTION
BIOMETRY

INSTITUTE AND LOCATION
NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.15	.15	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The advantages and disadvantages of using pre- or post- stratification methods in caries clinical trials were assessed. It was shown that, provided one has knowledge of one or more variables which are correlated with caries increments, the advantages of using a stratification method dominate, and therefore, stratification methods ought to be used. In those instances, not that commonly encountered, where the relationship can be assumed to be linear, the analysis of covariance performed just as well.

It was also shown that, provided the imbalance among cells is not too severe, both the additive and interaction models were robust, i.e., performed well even though the underlying assumptions needed for such analyses were unmet.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00364-02 EODP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Small Sample Behavior of the Standard Deviation for the Kaplan-Meier Estimator

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

LI, SHOU-HUA   STATISTICIAN (Vis.)   EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

EPIDEMIOLOGY BRANCH

SECTION

BIOMETRY

INSTITUTE AND LOCATION

NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.10	.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects   ☐ (b) Human tissues   ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We conduct Monte Carlo experiments under the model of random censorship to investigate the small sample behavior of different standard deviation formulas for the Kaplan-Meier estimator. The simulation studies include the Greenwood formula, Peto's formula and an alternative modified Greenwood formula. The results of the simulation showed that the Greenwood formula underestimated the true variance more than 90% of the times. Peto's formula overestimated the true variance when the survival rates were less than or equal to 0.5. The accuracy of the three different variance estimators is investigated using the relative bias, mean square error and confidence coefficient criteria. The absolute value of the relative bias for the modified Greenwood formula was the smallest among all three different variance estimators. On the basis of the mean square error criteria, the differences were negligible between the three different variance estimators. The Greenwood formula did not approach the confidence coefficient 0.95 in any censored observation. The confidence coefficient of Peto's formula was closer to 0.95 than the other two variance estimators. The sample sizes, censorship levels and distributions of the random variable all influenced the accuracy of the variance estimators.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00387-01

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Relationship between Specific Microorganisms in Saliva and Dental Caries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert	Statistician	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology

SECTION
Biometry

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.80	.40	Technical .40

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Potential associations between the levels of specific microorganisms in saliva and the level of dental caries in U.S. adolescents were investigated. Initial findings indicate that there is a positive correlation between the level of S. mutans and Lactobacillus in saliva and the prevalence of dental caries in this study population. The caries prevalences for participants having high levels of bacteria in saliva were 1.5 DMF surfaces higher than the corresponding prevalence for those having low levels of bacteria in saliva.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00388-01

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of a New Surface Index of Fluorosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

KINGMAN, ALBERT	STATISTICIAN	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
EPIDEMIOLOGY

SECTION
BIOMETRY

INSTITUTE AND LOCATION
NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.10	.10	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new index for scoring dental fluorosis, denoted by TSIF, recently developed at the NIDR, was evaluated for reproducibility by the two examiners who performed the fluorosis examinations. Each examiner was able to reproduce his fluorosis diagnoses upon repeated evaluation of a subsample of participants. The examiners experienced some difficulty in agreeing between themselves, especially in making the differentiation between the presence or absence of the very mild form of fluorosis.

In spite of the difference between examiners, this index was able to detect statistically different levels of fluorosis between groups having optimal, 2, 3 and 4 times optimal levels of fluoride in their water supplies, after adjusting for examiner differences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00389-01 EODP

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tooth Eruption Patterns of U.S. Schoolchildren, 1979-80

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

SMITH, JACQUELINE I.	STATISTICIAN	EODPP, NIDR
BRUNELLE, JANET A.	CHIEF, BIOMETRY SECTION	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
EPIDEMIOLOGY

SECTION
BIOMETRY

INSTITUTE AND LOCATION
NIDR, NIH, BETHESDA, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.25	.25	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Caries attack rates for permanent teeth of children are greatly influenced by the duration of exposure to risk. To better access caries susceptibility, it was decided to determine the eruption patterns of the children being studied. Using the dental findings of 37,066 children examined during the 1979-80 National Dental Caries Prevalence Survey, an estimation of the time of eruption for permanent teeth was made for children ages 4-18. Calculations were made of the percentages of children having each tooth at each age. Ages for the survey were rounded to the nearest birthdate. Eruption patterns were calculated for each upper and lower tooth by sex and race. Probit analysis was used to establish time of eruption and corresponding confidence limits.

The age at eruption of mandibular teeth was usually earlier than maxillary teeth, though some teeth, such as 2nd bicuspids, erupted at about the same time in both arches. As expected, there is a definite hierarchy of eruption by race and sex, with teeth erupting earlier in females than in males and in blacks earlier than in whites. The largest difference in age of eruption occured in upper and lower cuspids, which erupted approximately one and a quarter years earlier in black females than in white males.

Comparing our results with past studies of white children in the U.S., it appears that the teeth of present day white children are erupting earlier.

Annual Report of the Field Studies Section
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Field Studies Section was established during the year to conduct epidemiological studies on the broad range of oral disease. One function of this Section will be to collect data on the distribution and prevalence of oral disease and to monitor changes over time. A major effort in this regard has been initiated. In collaboration with the Biometry Section, a contract entitled "Epidemiologic Survey of Oral Health in Adults" was awarded on July 1, 1984. The primary purpose of the adult survey is to establish the prevalence of coronal caries, root surface caries and periodontal destruction in the adult population of the U.S. This survey will establish a baseline to permit future detection of changes and will provide estimates of current treatment needs of employed adults and non-institutionalized retired seniors. After completion of all calibration procedures and pilot studies, examination of the study population will begin by February 1985. The examination phase is expected to run for 12-16 months and will be conducted in the seven geographic regions utilized in the survey of dental caries prevalence in U.S. children, which was completed in 1980.

Another project has been initiated to investigate the prevalence of horizontal bone loss and its utility as a predictor of susceptibility to chronic periodontitis in an adolescent population of American Indians. Chronic adult periodontitis may frequently have its onset in adolescence but is rarely diagnosed until much later. If, indeed, significant numbers of teenagers do exhibit early signs of periodontitis and a simple, effective screening procedure could be devised, an important new approach to the diagnosis and treatment of periodontal disease would be available. Preliminary preparations for this study have been completed and data collection is scheduled to begin in October 1984.

A study to compare the anticaries effectiveness of two NaF/MFP dentifrices at fluoride concentrations of 1000 and 2500 ppm with a control MFP dentifrice of 1000 ppm F is being conducted under contract with the State University of New York at Stony Brook. This study began in 1981 and will terminate in 1985. The first year findings showed no differences between the three dentifrices. Second year data have not yet been analyzed; third year final examinations will be conducted in the spring of 1985.

To investigate the efficacy of sodium fluoride mouthrinsing in preventing dental caries in adults, a study was initiated in 1983 under a contract with the State University of New York at Stony Brook. This three-year clinical study of 1200 adults is being conducted under double blind conditions. Because of initial delays in assembling the study sample, the first year follow-up examination has not been completed at this time and no results are available to report.

Another study conducted by the Eastman Dental Center is designed to determine if the effectiveness of postnatal fluoride supplementation can be enhanced by prenatal administration of fluoride. A total of 1200 pregnant women and their subsequent offspring will participate in the study. At this time approximately 500 mothers have been enrolled. The children of the expectant mothers are to be examined for dental caries at 3 1/2 and again at five years of age; thus the study will not be completed until 1991.

A contract with Colgate-Palmolive Company examining the relationship between tooth fluoride and dental caries was completed during the year. It was concluded that, although fluoride levels in enamel are related to caries, numerous other factors are involved which mask the relationship. Thus, enamel fluoride is not a useful clinical predictor of caries rates. Removal of the outer enamel layer to eliminate the effect of local fluoride did not improve the correlation.

Other studies of periodontal disease are now in the active planning stage, including one concerning chronic periodontitis and tooth loss in adult twins. The Medical Follow-up Agency of the National Academy of Sciences-National Research Council maintains a register of World War II veteran twins. Potential twin samples are presently being compiled for several metropolitan centers in which adequate dental facilities exist.

Report of the Microbial Systematics Section
National Institute of Dental Research
October 1, 1983 - September 30, 1984

The Microbial Systematics Section is charged with establishing a data bank for information describing diverse strains of microorganisms. Special emphasis is placed on the human oral microbiota. For this purpose, collaborative projects are on-going with microbiologists distributed throughout the world.

At present there are tens of thousands of scientists, physicians, public health personnel, and others involved in some aspect of microbiology. The number of microbial strains isolated, characterized, and (in many cases) preserved, by individuals runs into the millions. Hundreds of millions of bits of information have been developed on these strains. However, these data are not resident in a single, centrally located system, permitting rapid and efficient utilization. Because of the large volume of information involved and because, in several applications such as classification and identification, mathematical manipulations of the data are required, electronic processing of these data is necessary.

In collaboration with personnel of the American Type Culture Collection, the Food and Drug Administration (FDA); the Centers for Disease Control (CDC), the Environmental Protection Agency, the Veteran's Administration and numerous academic microbiologists, strain data are being entered into the data bank which provides such services as: data on specific organisms and/or groups of organisms, location of strains with special characteristics, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids for research purposes, aids in quality control of tests, methods, and laboratories, and communication of data via common format.

Data files of primary data on a large number of microorganisms found in the oral cavity and related types are established. These files provide a resource for asking both ecological and epidemiological questions of interest in dental research.

Programs have been developed and tested to enter, retrieve, and analyze the data in a variety of ways for epidemiological, diagnostic, taxonomic, ecological, etc., uses. The long term goal is to establish a world-wide data bank at a series of cooperating centers. As experience grows, improved programs are being designed and implemented to form a computer utility for microbiological data management and analysis. The utility is termed the Microbial Information System (MICRO-IS). The MICRO-IS is in the initial stages of design for adaptation to use in micro- and minicomputer environments. By providing these programs, access to strain data world-wide will be possible under standard conditions.

The system of coding descriptors for computer input of information on bacteria, protozoa, fungi, algae, and its current extension to hybridomas has gained international attention and is of consequence to various components of NIH as well as other agencies in the Public Health Service (specifically FDA and CDC) and the National Science Foundation. The coding system has been

adopted as the standard coding method for microbial data by FDA, the World Data Center of the World Federation for Culture Collections, and the Japanese Federation of Culture Collections. The Committee on Data for Science and Technology (CODATA) of the International Council of Scientific Unions has agreed to sponsor the publication of the coding system for world-wide distribution.

CODATA has established a Task Group on a Hybridoma Data Bank for the purpose of establishing an international data bank on hybridoma and monoclonal antibody information. The Task Group is collaborating with the MSS in extending the microbial coding system to hybridomas using the Micro-IS programs. Two staff members of NIH (Dr. M.I. Krichevsky, MSS, NIDR and Dr. B. Janicki, National Institute of Allergy and Infectious Diseases) are members of the Task Group. The World Health Organization Immunology Program has volunteered staff for abstracting literature. The staff establishing the hybridoma data base is located at the American Type Culture Collection.

Extensive files of descriptions of filamentous and pleomorphic organisms are being assembled. The files cover all the described types of Mycobacteria, blend into the Nocardi, then through the actinomycetes (especially a unique set on oral isolates), and finally, Bacterionema. An extensive cooperative study, covers the oral pleomorphic bacteria (many of which are associated with disease). The study will provide a standard set of well characterized bacteria for the Dental Research community. The data from this study has been incorporated into the files on pleomorphic organisms. These files are being actively analyzed in collaboration with the submitters of the data as well as numerical taxonomists to revise the badly confused taxonomic relationships of these bacteria. Such revision is necessary to avoid the misidentification (leading to erroneous epidemiological conclusions) which are found in some recent dental research literature.

Other files on non-filamentous oral organisms (streptococci, lactobacilli, veillonella, etc.) are being constructed to study correlations among caries activity, phenetic span of characters, serology, source of isolation, and host descriptions.

One of the long term goals in establishing all these files is the establishment of probability tables to allow computer-aided probabilistic identification of oral isolates. Probability matrices, for on-line identification of bacteria (including Gram negative rods, lactobacilli, streptococci, bacilli, etc.) have been constructed. They are available to research workers for use.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DS 00044-14 EODP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Handling of Microbial Strain Information by Computers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Krichevsky, Micah I.	Research Chemist	NIDR, MSS
Love, Leslie L.	Information Specialist	NIDR, MSS

COOPERATING UNITS (if any)

see attachment

LAB/BRANCH

Epidemiology

SECTION

Microbial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.70	1.00	.70

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Microbial strain data are being entered into a data bank to provide: data on specific organisms, identification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids, aids in quality control of tests, methods, and laboratories, and communication of data via common format. Data files of primary data on microorganisms found in the oral cavity and related types are established, providing a resource for asking both ecological and epidemiological questions in dental research. Coding conventions have been developed to relate oral clinical parameters with the incidence and distribution patterns of specific microflora. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

Programs are being developed to enter, retrieve, and analyze the data for epidemiological, diagnostic, taxonomic, and ecological uses. The long term goal is to establish a world-wide data bank at a series of cooperating centers. The original bacterial system is being expanded to include the algae, yeasts, molds, protozoa, and hybridomas.

COOPERATING UNITS:
V. Jones, Food and Drug Administration
F. Benedict, Food and Drug Administration
R. Gherna, American Type Culture Collection
R. Good, Centers for Disease Control
V. Dowell, Centers for Disease Control
M. Segal, Environmental Protection Agency
L. Wayne, Veterans Administration
V. Sutter, Veterans Administration
R. Atlas, University of Louisville
S. Socransky, Forsyth Dental Center
M. Newman, UCLA
S. Holt, University of Massachusetts
V. Levy-Frebault, Pasteur Institute
H. Sugawara, Institute for Chemical and Physical Research

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00250-07 EQDP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Algorithms for Microbial Systematics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walczak, Cynthia A.	Computer Scientist	NIDR, MSS
Krichevsky, Micah I.	Research Chemist	NIDR, MSS
Mercer, Paula	Computer Programmer	NIDR, MSS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology

SECTION

Microbial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
2.18	2.08	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Algorithms are being developed and tested for aiding in numerical taxonomy of feature by strain matrices too large to be analyzed by existing programs. Both segmentation and heuristic approaches are being investigated.

A program has been developed and is being tested to compare and evaluate methods and/or laboratories when characterizing the same set of strains. The usual statistical packages are not useful because of the predominantly binary (i.e., discontinuous) nature of the data. The algorithm allows comparison of tests or laboratories at the levels of the individual strain (with replicable determinations), species, genus, and overall set for determination of test method equivalences and/or inter-laboratory consistancy.

Computer graphic algorithms are being tested to aid microbiologist in visualizing individual similarities as well as hierarchical group memberships among strains.

Annual Report of the Clinical Trials Section
NATIONAL INSTITUTE OF DENTAL RESEARCH

Several reports show that a decrease in the prevalence of dental caries has occurred in the United States in the last several years. However, a sizable amount of dental decay still exists. Because the use of fluorides remains the most effective method of caries control, the Clinical Trials Section (CTS) continues to devote a major portion of its research effort to developing combinations of fluoride agents and procedures to their maximum anticaries potential.

To determine the caries preventive effect of a combination of some of the most feasible self-administered fluoride procedures, the CTS began a long-term study in October 1972 in Nelson County, Virginia. Children in the County's schools, under their teachers' supervision, ingest daily a 1 mg. F tablet and rinse weekly with a 0.2% NaF solution; a fluoride dentifrice is provided for ad libitum use at home. Findings, in 1983, after eleven years of the program, showed an overall 65% lower prevalence of dental caries and a reduction of 90% in mesiodistal surfaces compared with findings of children examined before the program began.

Although all types of surfaces benefited from the fluoride regimen in Nelson County, pit and fissure surfaces were the least protected. In fact, more than 90% of the total DMFS count in 1983 occurred in pit and fissure tooth surfaces. To prevent this remaining type of decay a sealant program was added to the ongoing fluoride program in Nelson County in 1984. Newly erupted teeth of children in selected grades will be sealed each year for a period of four years. It is likely that the combined fluoride-sealant program will virtually eliminate decay among children in Nelson County.

Besides prompting an evaluation of sealants with fluorides, the Nelson County study has spurred interest in isolating the additive effects of fluorides. Because all study participants received all of the protective regimens in the Nelson County study, only the total effect could be evaluated. To determine the caries preventive benefits specifically from daily fluoride supplements, weekly fluoride rinsing and the two methods combined, a long-term study was initiated in 1981 in Springfield, Ohio. First follow-up examinations were made in 1983. Analysis of the two-year data shows that children who received both fluoride procedures had the lowest incremental caries scores, followed by those who ingested fluoride tablets; subjects who used only the fluoride rinse had the largest caries increment. The same relative order of effectiveness was found in both primary and permanent teeth. However, none of the differences among the groups reached statistical significance at this early stage of the study.

Two protocols for new studies to be conducted as direct operations by the CTS are currently in preparation. One is for a follow-up survey of children who participated in an epidemiologic study carried out by NIDR in 1980 in four areas of Illinois that have varying levels of natural fluorides (optimum, 2X, 3X and 4X) in their drinking water supplies. The same communities will be revisited in 1985 to examine the same children who were 8-10 years of age in 1980. These children will be 13-15 years old at the time of the follow-up survey; their teeth that had fluorosis in 1980 will be reexamined for any changes in the severity of the condition. The findings will help determine whether the lower prevalence of fluorosis in the older age groups observed during the 1980 survey resulted from abrasion or remineralization or from a lower intake of fluoride. A group of children 8-10 years of age will also be examined in the 1985 survey to determine if there are secular changes in fluorosis occurring in the Illinois communities. In addition, the prevalence and extent of gingivitis will be assessed in older children to provide information on the effect of optimal and above-optimal levels of fluoride in the drinking water on the health of the periodontium.

The other protocol being developed is designed to determine the caries inhibitory effects of weekly sodium fluoride mouthrinses in a non-fluoridated and in an optimally fluoridated community. Many studies have demonstrated the unquestionable value of fluoride mouthrinsing in reducing dental caries, but the majority of these clinical trials were conducted when the prevalence of dental decay in the United States was higher than it is today. Because of the current reduction in caries prevalence, it can be argued that fluoride mouthrinsing is no longer as effective as it once was. Currently, approximately 12 million school children are participating in fluoride rinse programs. Information obtained from this study will help determine whether school fluoride rinse programs should continue to be recommended as an effective public health measure.

Critical to any rationale for including a maximum contaminant limit for fluoride in EPA's National Interim Primary Drinking Water Regulations is whether fluoride, at concentrations found in U.S. water supplies, constitutes a hazard to dental health. While there is no evidence to show that children's dentition are adversely affected, data on the long-term sequelae of dental fluorosis in adults are completely lacking. To gain information about this important public health issue, the NIDR contracted with the University of Michigan to compare the oral health of lifetime adult residents of an area with severe endemic dental fluorosis with that of residents in an area with optimally fluoridated drinking water. Cross-sectional examinations were conducted by the Michigan examiners in July 1984. Several variables of oral health including, dental fluorosis, dental caries, tooth loss and periodontal disease were assessed. Findings of the two communities will be compared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00070-12 EODP

PERIOD COVERED

October 1, 1983 to September 30, 1984 CT 0600045

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Combined self-applied fluorides and sealants for caries prevention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Horowitz, Herschel S.	Chief, Clinical Trials Section	NIDR, DPB, EODP
Heifetz, Stanley B.	Senior Field Investigator	NIDR, DPB, EODP
Meyers, Rhea J.	Clinical Trials Administrator	NIDR, DPB, EODP
Driscoll, William S.	Senior Field Investigator	NIDR, DPB, EODP
Li, Shou-Hua	Visiting Scientist	NIDR, EB, EODP
Bellafiore, Carolyn	Secretary	NIDR, DPB, EODP

COOPERATING UNITS (if any)

Nelson County, Virginia Public School System

LAB/BRANCH

Disease Prevention Branch

SECTION

Clinical Trials

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.00	.70	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Baseline dental examinations were conducted in October 1972, on approximately 2200 children (grades 1-12). All participants in grades K-6 chew daily in school under teacher supervision a sodium fluoride tablet containing 1 mg F, and swish and swallow the resultant solution. On a scheduled basis, a fluoride-containing dentifrice and toothbrushes are distributed to the children for use at home. Kindergarten classes were invited to participate in the program beginning in the 1976-77 school year. Children in the 7th and 8th grades in Nelson County's junior high school began to participate in the program in the fall of 1978 and 1979, respectively. Beginning in the fall of 1980, high school students in Nelson County began to participate only in the tablet and dentifrice components of the program. For the period covered by this report, children in grades K-12 were participating. In January 1984, a sealant program was added to the ongoing fluoride program. Newly erupted teeth of children in selected grades were sealed with a pit and fissure sealant. The program will run for five years. The combined preventive program has the potential of virtually eliminating dental caries in Nelson County.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00277-05 EODP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Dental caries and fluorosis in areas with different water fluoride concentrations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horowitz, Herschel S.	Chief, Clinical Trials Section	NIDR, DPB, EODP
Driscoll, William S.	Senior Field Investigator	NIDR, DPB, EODP
Meyers, Rhea J.	Clinical Trials Administrator	NIDR, DPB, EODP
Kingman, Albert	Statistician (Health)	NIDR, EB, EODP
Bellafiore, Carolyn	Secretary	NIDR, DPB, EODP

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention Branch

SECTION
Clinical Trials

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.22	.20	.02

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

In April 1980, a cross-sectional survey was made of the prevalence of dental fluorosis and dental caries in seven communities in Illinois that had different concentrations of naturally-occurring fluoride in their public water supplies. The communities were grouped into four categories according to the approximate relation of their actual fluoride concentration to the recommended optimal fluoride concentration for the area (1, 2, 3 or 4 times optimal). The study population consisted of 807 school children, ages 8-16, who had lived continuously from birth in their respective communities and had always used the community water supply as their primary source. Dental caries was assessed with the DMFS index and dental fluorosis was measured with Dean's Index and with a newly developed Tooth Surface Index of Fluorosis (TSIF). Fluorosis was assessed independently in each child by each Index. In addition, color photographs of the teeth of some children were taken to depict varying degrees of fluorosis. In April 1982, 317 children from four communities in Iowa with negligible concentrations of fluoride in their drinking water were examined for comparison with the children in Illinois. Two reports of findings based on the Illinois data have been published. One presented basic findings on dental caries and dental fluorosis as measured with Dean's Index; the other presented findings based on the new Tooth Surface Index of Fluorosis. A third report, presenting the Iowa data along with supplementary findings from Illinois, is currently being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00310-04 EODP

PERIOD COVERED
October 1, 1983 to September 30, 1984 CT 0600144

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Evaluation of fluoride mouthrinsing and fluoride tablets when used separately and in combination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Horowitz, Herschel S.	Chief, Clinical Trials Section	NIDR, DPB, EODP
Heifetz, Stanley B.	Senior Field Investigator	NIDR, DPB, EODP
Meyers, Rhea J.	Clinical Trials Administrator	NIDR, DPB, EODP
Li, Shou-Hua	Visiting Scientist	NIDR, EB, EODP
Bellafiore, Carolyn	Secretary	NIDR, DPB, EODP

COOPERATING UNITS (if any)
Springfield, Ohio, Public and Non-Public Schools

LAB/BRANCH
Disease Prevention Branch

SECTION
Clinical Trials

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.89	.56	.33

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Approximately 1700 kindergarten and first grade children were randomly assigned to one of the following groups:

Group I - Rinses once every week in school with a 0.2% sodium fluoride solution.
Group III - Ingests once a day in school a sodium fluoride tablet containing 1 mg of fluoride.
Group II - Carries out the regimens for both Group I and Group III.

The method of assignment resulted in three comparable groups each containing about 560 children. Participants carry out their assigned treatments in classrooms under the close supervision of a teacher. Treatments will be administered for a minimum of eight and nine years for first grade and kindergarten, respectively. Baseline dental examinations were conducted in September 1981. The prescribed treatments were initiated shortly after the examinations were completed.

In September 1982 and 1983, the second and third years of treatments, respectively, were begun in the classrooms. First follow-up dental examinations were made in October 1983 of 1,165 participants who were in grades 2 and 3. Two-year data are currently being analyzed.

Annual Report of the Laboratory Methods Section
NATIONAL INSTITUTE OF DENTAL RESEARCH

The realignment of the National Caries Program effected an expansion of research involving dental conditions in addition to dental caries, and resulted in the establishment of the Laboratory Methods Section. The work emanating from this period of transition has been both a continuation and completion of ongoing projects involving dental caries epidemiology, prevention and control, as well as more recent efforts in the investigation of aspects of periodontal diseases.

The intraoral fluoride-releasing device developed by Southern Research Institute for the National Caries Program continues to be of significant interest. Studies in rats repeatedly indicate a beneficial anticaries action through topical effects. This effect appears to exist for a significant time period after the device is removed. We plan to pursue this mode of fluoride delivery by: 1) using different concentrations of fluoride with various release rates; 2) improving the design of the device and its method of attachment; and 3) conducting small clinical trials using the device.

Increasing concern over the relationship between fluorosis and water fluoride concentration, fluoride in toothpastes/mouthwashes and fluoride in the food chain has led to our reevaluation of some microanalytic methods for plaque fluoride analysis. Samples of dental plaque from monkeys exhibited similar levels of fluoride by heat mediated and silane mediated diffusion methods. However, the fluoride appeared to be unevenly distributed in dental plaque and varied in amount from week to week.

Investigations of octenidine, a potential antiplaque, anticaries and antigingivitis agent, have been in progress in our laboratory since 1977. Studies in rats and monkeys indicate it may afford sustained antimicrobial action. Results from small short-term clinical trials have been encouraging. Activity will continue in assessing the effectiveness of this agent. Also under development is methodology (HPLC) for detection of various agents in dental plaque, saliva and other fluids.

Limited efforts have begun to investigate an animal model for the study of periodontal disease. Considerable time has been devoted to establishing a collection of microorganisms considered to be associated with periodontal diseases. Methodology for production of antisera to many of the bacteria is being developed and assessed. These antisera will be valuable tools in planned epidemiologic investigations.

Considerable time has been devoted to assessing non-sucrose sweetening agents and dental caries in rats. Various regimens and supplemental diets have been investigated for the purpose of refining our animal model.

Transmission of Streptococcus mutans among individuals has been of significant interest to our program. Several experiments have been performed to establish an animal model (rats): 1) to study the various factors that affect transmission; and 2) to evaluate procedures that may inhibit transmission and subsequent establishment/colonization of specific oral microorganisms.

In an attempt to elucidate predictive factors, section members are developing and evaluating various parameters of periodontal conditions including crevicular flow, enzyme activity, and microbiologic assessment.

Section personnel continue to participate in planning, conducting and/or assisting in epidemiologic surveys conducted by the Epidemiology and Oral Disease Prevention Program. This necessitates a continual evaluation of existing methodology for preservation, shipment, storage and subsequent assay of dental plaque and saliva for several factors.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00112 11 EODPP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Preclinical screening of anticaries agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Shern, Roald J.	Principal Investigator	EODPP LMS NIDR
Adderly, Donna D.	Chemist	EODPP LMS NIDR
Kennedy, John B.	Biologist	EODPP LMS NIDR
Little, Wayne A.	Microbiologist	EODPP LMS NIDR
Mirth, Dale B.	Research Chemist	EODPP LMS NIDR
Monell-Torrens, E.	Bio Lab Technician	EODPP LMS NIDR
Stiles, Horace M.	Chief, LM Section	EODPP LMS NIDR

COOPERATING UNITS (if any)

Clinical Investigations and Patient Care Branch, NIDR (Michael W. Roberts) American Dental Association Health Found. National Bureau of Standards, Gaithersburg, Md. (W.E. Brown and L.C. Chow)

LAB/BRANCH

Disease Prevention Branch

SECTION

Laboratory Methods Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.08	0.63	0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The principal objectives of this project are part of the ongoing effort identifying antiplaque and anticaries agents suitable for short-term clincal investigation, as well as to develop methods for assessing the clinical potential for these agents.

Data from the rat studies indicated that the levels of enamel bound fluoride exhibit: (1) a negative association with the scores for dental caries; and (2) a negative trend with pH decreases in dental plaque following a sucrose rinse.

Data from the studies in monkeys indicate that oral rinses with solutions containing octenidine will reduce the quantity and pathogenic potential of existing dental plaque. Methods are being developed for measuring responses related to the various periodontal diseases.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00234 07 EODPP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Develop method of intraoral telemetry of various ions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Shern, Roald J.	Principal Investigator	EODPP LMS NIDR
Stiles, Horace M.	Chief, LM Section	EODPP LMS NIDR
Kennedy, John B.	Biologist	EODPP LMS NIDR
Smith, Jacqueline I.	Statistician (Health)	EODPP LMS NIDR

COOPERATING UNITS *(if any)*

Biomedical Engineering and Instrumentation Branch (T.L. Talbot, H.W. Tipton)
Commissioned Officers Dental Clinic (A.D. Guckes)

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.37	0.27	0.10

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory is using both intraoral and extraoral methods of measuring the pH of dental plaque following ingestion of various test foods. Time courses for pH following sucrose ingestion are similar except the extraoral method detects the drop more rapidly than does the intraoral method. In past studies (here and elsewhere) the indwelling glass pH electrode often failed to respond properly because of moisture leakage of the intraoral connector. This problem has been eliminated by incorporating a gas tube with the wire connector. The tube emits a stream of nitrogen which keeps critical electronic junctions moisture-free and well-insulated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00281 05 EODPP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of oral fluids using high performance liquid chromatography (HPLC)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mirth, Dale B.	Research Chemist	EODPP DPB NIDR
Adderly, Donna D.	Chemist	EODPP DPB NIDR
Li, Shou-H.	Visiting Statistician	EODPP EB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention Branch

SECTION

Laboratory Methods

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN YEARS	PROFESSIONAL	OTHER
0.63	0.12	0.51

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent advances in high performance liquid chromatography (HPLC) column technology have made it possible to rapidly analyze protein samples using HPLC. The purpose of the present project was to evaluate high performance liquid chromatography as a means of analyzing biological fluids such as saliva and plaque fluid and to see if it was feasible to quantitate the secretory immunoglobulin A (s-IgA) in saliva by HPLC.

This project was carried out using a Waters Associates HPLC system.

The results suggest that HPLC can be used to quantitate constituents of saliva such as s-IgA and these results compare favorably to results from other commonly used methods. The HPLC method will be examined as a means to rapidly isolate s-IgA from saliva.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00282 05 EODPP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Anticaries evaluation of an intraoral fluoride-releasing device in rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mirth, Dale B.	Research Chemist	EODPP DPB NIDR
Adderly, Donna D.	Chemist	EODPP DPB NIDR
Monell-Torrens, Esteben	Bio Lab Tech. (Animal)	EODPP DPB NIDR
Canniff, Edward T.	Dental Costep In-Serv	EODPP EB NIDR
Li, Shou-Hua	Visiting Scientist	EODPP BS NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention Branch

SECTION
Laboratory Methods Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.97	0.42	0.55

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous in vitro and in vivo studies have shown that an intraoral fluoride-releasing device developed by the Southern Research Institute for the National Institute of Dental Research will deliver fluoride at a steady rate for up to six months. This project will evaluate the anticaries effect and mechanism of action of the fluoride-releasing device in rats. Results have shown that rats that had an intraoral fluoride-releasing device in their mouth developed significantly fewer caries on all surfaces than untreated or placebo treated animals. The data indicate that the marked caries reduction produced by the fluoride-releasing device was due to topical effects of fluoride. Additional data suggest that the cariostatic effects of the intraoral fluoride-releasing device may persist for a significant time period after the device is removed from the mouth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00319 04 EODPP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
A Simple Microdiffusion Technique for Measuring Fluoride in Biological Samples

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Shern, Roald J.	Principal Investigator	EODPP DPB NIDR
Kennedy, John B.	Biologist	EODPP DPB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS (if any)

Disease Prevention Branch

LAB/BRANCH
Laboratory Methods

SECTION
NIDR, NIH, Bethesda, Maryland

INSTITUTE AND LOCATION

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.41	0.10	0.31

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previously this laboratory reported that various microanalytic methods detected different levels of fluoride in aliquots of plasma from the same sample. Currently we are assessing fluoride levels in monkey dental plaque: (1) aliquots of pooled dental plaque; (2) samples from maxillary and mandibular teeth collected over several weeks. Both the heat mediated and silane mediated methods detected similar levels of fluoride. The fluoride appeared to be unevenly distributed in dental plaque and varied in amount from week to week.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00325 04 EODPP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Streptococcus mutans transmission in rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Stiles, Horace M.	Chief, LM Section	EODPP	DPB	NIDR
Monnell-Torrens, Esteban	Laboratory Technician	EODPP	DPB	NIDR
Gomez, Irma	Laboratory Technician	EODPP	DPB	NIDR
Brunelle, Janet A.	Chief, B Section	EODPP	EB	NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP	DPB	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention Branch

SECTION

Laboratory Methods

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.62	0.22	0.42

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Results from Phase 1 of this project indicated that transmission of S. mutans 6715 occurred among rats consuming 0, 0.1, 1 and 56 percent sucrose in their diets. Generally, increased levels of infection were associated with increases in dietary sucrose concentrations. Recipient rats fed 0-5 percent sucrose showed no difference in level of infection or any difference in smooth surface caries scores; those fed 56 percent sucrose attained the highest level of infection and also the highest caries scores of any group.

Phase 2 of this project examined the transmission of S. mutans and subsequent caries development at intervals among rats consuming 56 percent sucrose in their diets. The data indicate that although the original inoculum for the donors was 10-8 to 10-9 cells/ml, their infection levels increased with time and appeared to plateau around day 40. Transmission from donors to recipients occurred as early as day 1 (1 pair) and was delayed as long as 17 days (3 pairs). Transmission was detected in over 50 percent of the recipients by the 10th day and by day 17 approximately 90 percent of the recipients were infected. Mean smooth surface caries scores appeared to be more strongly associated with length of infection rather than the infection level, i.e., regardless of the level of infection, mean caries scores increased with time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00365 02 EODPP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of a non-sucrose sweetener on rat caries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Monnell-Torrens, Esteban	Laboratory Technician	EODPP DPB NIDR
Gomez, Irma M.	Laboratory Technician	EODPP DPB NIDR
Brunelle, Janet A.	Chief B Section	EODPP EB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention Branch

SECTION

Laboratory Methods

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.83	0.22	0.61

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acesulfam K (AK) is a non-sucrose sweetener developed by Hoechst Corporation. According to its manufacturer it is 300-350 times as sweet as sucrose. Because of its potential use as a sucrose substitute by individual and industrial consumers, its cariogenic potential was investigated.

The experiment consisted of three groups of rats (10/group) which were fed one of the following diets: Group One (Suc) -NIH Diet 2000 (56 percent sucrose); Group Two (AK) -NIH Diet 2114 (56 percent raw corn starch) plus 5 percent Acesulfam K; Group Three (RCS) Diet 2114 (56 percent raw corn starch). The experiment was performed using a Konig-Hofer progammable feeding machine which delivered 17 meals/17 hour period of the assigned diets. An additional 12 meals of diet 2114 was provided all three groups as a supplement. All animals were initially infected with S. mutans 6715. Oral infection levels were determined twice weekly. After 56 experimental days the rats were sacrificed. Mean smooth surface caries scores for the three groups were: Suc=13.4; AK=0; and RCS=5.9. It appears that AK has a low cariogenic potential in rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00371 02 EODPP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Antisera on Glucose Uptake by Streptococcus mutans 6715

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Little, Wayne A.	Microbiologist	EODPP DPB NIDR
Ciardi, Joseph E.	Research Chemist	LMI NIDR

COOPERATING UNITS *(if any)*

University of Oslo, Norway (Prof. Gunnar Rolla)

LAB/BRANCH

Disease Prevention Branch

SECTION

Laboratory Methods

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.22	0.01	0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study was to investigate the hypothesis that a protective mechanism of salivary antibody may be via interference with the glucose uptake system of acidogenic bacteria such as S. mutans. The effect of antisera to whole cells or cell wall components on glucose uptake by S. mutans 6715 was examined. Stationary phase 6715 cells were treated with test serum and incubated at 37 degrees C in the presence of 14-C -glucose. Samples were removed at timed intervals and measured in a liquid scintillation counter for radio-active uptake. Antisera to both whole cells and components known to be present on the surface of the cells reduced glucose uptake relative to normal serum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00390 01 EODPP

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Cariogenicity of palatinit in rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Monell-Torrens, Esteban	Laboratory Technician	EODPP DPB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Alexander, Catherine R.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention Branch

SECTION

Laboratory Methods

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.62	0.20	0.42

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Palatinit is a non-sucrose sweetener which is reported to be aproximately half the sweetness of sucrose. Because of its potential use as a sucrose substitute by individual and industrial consumers, its cariogenic potential should be determined.

The experiment consisted of four groups of rats (10/group) which were fed one of the following diets: Group 1 (Suc) - NIH Diet 2000 (56 percent sucrose); Group 2 (RCS) - NIH Diet 2114 (56 percent raw corn starch); Group 3 (P) - 56 percent raw corn starch replaced by 56 percent palatinit); Group 4 (P2) - 25 percent palatinit plus 31 percent raw corn starch. The experiment was performed using a Konig-Hofer progammable feeding machine which delivered 17 meals/17 hour period of the assigned diets. An additional 6 meals of diet 2114 was provided all four groups as a supplement. All animals were initially infected with S. mutans 6715. Oral infection levels were determined twice weekly. After 56 experimental days the rats were sacrificed. Data are presently being analyzed.

ANNUAL REPORT OF THE EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM NATIONAL INSTITUTE OF DENTAL RESEARCH

As of January 1984, the newly organized Epidemiology and Oral Disease Prevention Program began operation, staffed largely with personnel from the former National Caries Program. The E&ODP Program is charged with the conduct of descriptive and analytical epidemiologic studies of all oral diseases as well as the initiation of controlled clinical trials of potential disease-preventing agents and regimens.

During the few months of its existence, E&ODP staff have given highest priority to completing plans for a nationwide survey of the prevalence of oral disease in adults and the aged. This study will begin in February 1985, and will generate the most comprehensive set of data yet assembled on the dental conditions of U.S. adults. In addition, plans are well developed to repeat the national survey of the prevalence of caries in school children, completed in 1980. It is essential to continue to monitor caries levels in children to determine whether the downward trend in prevalence, observed during the 1970s, will continue.

Although many believe that the decline in dental caries is attributable to widespread use of fluorides, this hypothesis remains unproven, and requires further study. To that end, discussions have begun regarding a collaborative study of caries trends in West Germany, where fluoride programs have never been implemented.

Clinical trials are continuing on the effectiveness of a controlled release system for intraoral delivery of fluoride, on the effects of prenatal exposure to systemic fluorides and on the possibility that dental plaque can be controlled by the use of the antimicrobial, octenidine, as a mouthrinse.

New epidemiologic studies, designed during the year for implementation in FY 1985, include investigation of the prevalence of marginal loss of alveolar bone in adolescents and its possible value in predicting susceptibility to adult periodontitis. Further studies of the prevalence of enamel fluorosis are also planned, to continue to monitor levels of ingestion of fluorides from all sources. Thus far, data which have been collected suggest that, contrary to the concern of some, the prevalence of fluorosis has not increased in recent decades.

During the year, Dr. Jerry Niswander joined the Program staff as Chief, Epidemiology Branch, and is also serving as Acting Chief of the Field Studies Section. Ms. Mary Wolfe and Dr. Edith Morrison joined that Section as Epidemiologists.

The Associate Director was elected to the Executive Board of the European Organization for Caries Research and was the recipient of the PHS Superior Service Award. Dr. Herschel Horowitz, Chief, Clinical Trials Section, received the H. Trendley Dean Award in epidemiology from the International Association for Dental Research.

Annual Report of the Epidemiology Branch
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Epidemiology Branch applies epidemiologic and biometric methods to the study of distribution, cause, prevention, diagnosis and treatment of oral diseases. On abolishment of the National Caries Program, the Epidemiology Branch was created by combining the Biometry Section of the former Caries Prevention and Research Branch with the Microbial Systematics Section of the Intramural Programs. A third component, the Field Studies Section, was created de novo primarily with personnel from several areas of the NCP.

Under this new organization, the Biometry Section will continue to function in much the same manner as previously with the same staff conducting independent research as well as providing data processing and analysis services for the Branch and other programs of the Institute. Likewise, the Microbial Systematics Section continues much as before the reorganization. However, the expertise in microbiology and the capability for handling large files of microbiological data provide an important new resource for epidemiologic studies, particularly for studies of periodontal disease. The new Field Studies Section provides the data collecting resource but will draw heavily upon the other two sections for planning and design of its studies as well as data analysis.

The broad mandate of this new Branch offers many opportunities for new research activities which are presently being developed. Although dental caries will continue to be an area of concern, considerable emphasis is being placed on other oral conditions. The first priority is the development of an active program in periodontal disease. Several new studies have been initiated or are presently in the planning stage.

Annual Report of the Biometry Section
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Biometry Section has collaborated with members of other sections on two major clinical trials several related human and animal immunity studies, S. mutans transmission and caries prevalence studies, and animal studies of bacteria, anti-plaque agents and fluorides. It has also consulted on the design and analysis for three contracts and processed data from six clinical trial contractors.

Staff of the Biometry Section have also participated in the planning of four workshops/conferences on a broad range of topics: nutrition and caries; periodontal clinical trials; epidemiology principles; and caries prevalence, all of which will take place in the coming years. Two papers from the Caries Clinical Trial Workshop of May, 1983, "Stratification Methods in Caries Clinical Trials" and "Use and Misuse of Computer Programs in the Design and Analysis of Caries Clinical Trials," were published this year.

A major task now underway is the processing and tabulation of a two-part survey of oral health of Hispanics, conducted by the National Center for Health Statistics (NCHS). The first part, consisting of approximately 7000 comprehensive dental exams, has been furnished and returned to NCHS. The second part will be conducted until January, 1985. We are expecting to do a brief analysis of caries prevalence on the first and second parts when the survey weights have been added to the raw data tapes.

In conjunction with a clinical trial contract with the University of Michigan on diet and dental caries, a project is underway to analyze the relationship of certain microorganisms in saliva to caries levels in teenagers. The baseline data collected in Fall, 1982 indicated about a one and one-half surface difference between children with high and low levels of S. mutans in their saliva. Saliva from the second exam conducted in Spring, 1984 is being analyzed now and these positive relationships between caries and S. mutans appear to be quite strong again.

Besides consulting with the various laboratory and clinical investigators, we have been working on use of the growth curve model for analysis of microbiological changes in plaque collected over a period of time. This statistical model seems to describe the bacterial activity in a study of natural transmission of S. mutans in rats on various sucrose diets very well.

Using the data from the children's caries prevalence survey of 1979-80, tooth eruption patterns by age, sex and race for U.S. children were determined using probit analysis. It appears that white children may be erupting their teeth earlier than formerly.

Differences in eruption times by race and sex were found, with teeth erupting earlier in females than males and in blacks earlier than whites. The largest difference in age of eruption occurred in upper and lower cuspids, which erupted approximately one and a quarter years earlier in black females than in white males.

The caries prevalence survey data were also used to examine the DMFS prevalence differences between white children and those of other races. Overall, black children still seem to have lower caries prevalence, but differences are quite small. However, blacks and other non-white children in fluoridated cities average about one less DMFS surface than whites. In rural areas and non-fluoridated areas, there are little or no differences between the races.

Annual Report of the Laboratory Methods Section
NATIONAL INSTITUTE OF DENTAL RESEARCH

The realignment of the National Caries Program effected an expansion of research involving dental conditions in addition to dental caries, and resulted in the establishment of the Laboratory Methods Section. The work emanating from this period of transition has been both a continuation and completion of ongoing projects involving dental caries epidemiology, prevention and control, as well as more recent efforts in the investigation of aspects of periodontal diseases.

The intraoral fluoride-releasing device developed by Southern Research Institute for the National Caries Program continues to be of significant interest. Studies in rats repeatedly indicate a beneficial anticaries action through topical effects. This effect appears to exist for a significant time period after the device is removed. We plan to pursue this mode of fluoride delivery by: 1) using different concentrations of fluoride with various release rates; 2) improving the design of the device and its method of attachment; and 3) conducting small clinical trials using the device.

Increasing concern over the relationship between fluorosis and water fluoride concentration, fluoride in toothpastes/mouthwashes and fluoride in the food chain has led to our reevaluation of some microanalytic methods for plaque fluoride analysis. Samples of dental plaque from monkeys exhibited similar levels of fluoride by heat mediated and silane mediated diffusion methods. However, the fluoride appeared to be unevenly distributed in dental plaque and varied in amount from week to week.

Investigations of octenidine, a potential antiplaque, anticaries and antigingivitis agent, have been in progress in our laboratory since 1977. Studies in rats and monkeys indicate it may afford sustained antimicrobial action. Results from small short-term clinical trials have been encouraging. Activity will continue in assessing the effectiveness of this agent. Also under development is methodology (HPLC) for detection of various agents in dental plaque, saliva and other fluids.

Limited efforts have begun to investigate an animal model for the study of periodontal disease. Considerable time has been devoted to establishing a collection of microorganisms considered to be associated with periodontal diseases. Methodology for production of antisera to many of the bacteria is being developed and assessed. These antisera will be valuable tools in planned epidemiologic investigations.

Considerable time has been devoted to assessing non-sucrose sweetening agents and dental caries in rats. Various regimens and supplemental diets have been investigated for the purpose of refining our animal model.

Transmission of Streptococcus mutans among individuals has been of significant interest to our program. Several experiments have been performed to establish an animal model (rats): 1) to study the various factors that affect transmission; and 2) to evaluate procedures that may inhibit transmission and subsequent establishment/colonization of specific oral microorganisms.

In an attempt to elucidate predictive factors, section members are developing and evaluating various parameters of periodontal conditions including crevicular flow, enzyme activity, and microbiologic assessment.

Section personnel continue to participate in planning, conducting and/or assisting in epidemiologic surveys conducted by the Epidemiology and Oral Disease Prevention Program. This necessitates a continual evaluation of existing methodology for preservation, shipment, storage and subsequent assay of dental plaque and saliva for several factors.

Annual Report of the Clinical Trials Section
NATIONAL INSTITUTE OF DENTAL RESEARCH

Several reports show that a decrease in the prevalence of dental caries has occurred in the United States in the last several years. However, a sizable amount of dental decay still exists. Because the use of fluorides remains the most effective method of caries control, the Clinical Trials Section (CTS) continues to devote a major portion of its research effort to developing combinations of fluoride agents and procedures to their maximum anticaries potential.

To determine the caries preventive effect of a combination of some of the most feasible self-administered fluoride procedures, the CTS began a long-term study in October 1972 in Nelson County, Virginia. Children in the County's schools, under their teachers' supervision, ingest daily a 1 mg. F tablet and rinse weekly with a 0.2% NaF solution; a fluoride dentifrice is provided for ad libitum use at home. Findings, in 1983, after eleven years of the program, showed an overall 65% lower prevalence of dental caries and a reduction of 90% in mesiodistal surfaces compared with findings of children examined before the program began.

Although all types of surfaces benefited from the fluoride regimen in Nelson County, pit and fissure surfaces were the least protected. In fact, more than 90% of the total DMFS count in 1983 occurred in pit and fissure tooth surfaces. To prevent this remaining type of decay a sealant program was added to the ongoing fluoride program in Nelson County in 1984. Newly erupted teeth of children in selected grades will be sealed each year for a period of four years. It is likely that the combined fluoride-sealant program will virtually eliminate decay among children in Nelson County.

Besides prompting an evaluation of sealants with fluorides, the Nelson County study has spurred interest in isolating the additive effects of fluorides. Because all study participants received all of the protective regimens in the Nelson County study, only the total effect could be evaluated. To determine the caries preventive benefits specifically from daily fluoride supplements, weekly fluoride rinsing and the two methods combined, a long-term study was initiated in 1981 in Springfield, Ohio. First follow-up examinations were made in 1983. Analysis of the two-year data shows that children who received both fluoride procedures had the lowest incremental caries scores, followed by those who ingested fluoride tablets; subjects who used only the fluoride rinse had the largest caries increment. The same relative order of effectiveness was found in both primary and permanent teeth. However, none of the differences among the groups reached statistical significance at this early stage of the study.

Two protocols for new studies to be conducted as direct operations by the CTS are currently in preparation. One is for a follow-up survey of children who participated in an epidemiologic study carried out by NIDR in 1980 in four areas of Illinois that have varying levels of natural fluorides (optimum, 2X, 3X and 4X) in their drinking water supplies. The same communities will be revisited in 1985 to examine the same children who were 8-10 years of age in 1980. These children will be 13-15 years old at the time of the follow-up survey; their teeth that had fluorosis in 1980 will be reexamined for any changes in the severity of the condition. The findings will help determine whether the lower prevalence of fluorosis in the older age groups observed during the 1980 survey resulted from abrasion or remineralization or from a lower intake of fluoride. A group of children 8-10 years of age will also be examined in the 1985 survey to determine if there are secular changes in fluorosis occurring in the Illinois communities. In addition, the prevalence and extent of gingivitis will be assessed in older children to provide information on the effect of optimal and above-optimal levels of fluoride in the drinking water on the health of the periodontium.

The other protocol being developed is designed to determine the caries inhibitory effects of weekly sodium fluoride mouthrinses in a non-fluoridated and in an optimally fluoridated community. Many studies have demonstrated the unquestionable value of fluoride mouthrinsing in reducing dental caries, but the majority of these clinical trials were conducted when the prevalence of dental decay in the United States was higher than it is today. Because of the current reduction in caries prevalence, it can be argued that fluoride mouthrinsing is no longer as effective as it once was. Currently, approximately 12 million school children are participating in fluoride rinse programs. Information obtained from this study will help determine whether school fluoride rinse programs should continue to be recommended as an effective public health measure.

Critical to any rationale for including a maximum contaminant limit for fluoride in EPA's National Interim Primary Drinking Water Regulations is whether fluoride, at concentrations found in U.S. water supplies, constitutes a hazard to dental health. While there is no evidence to show that children's dentition are adversely affected, data on the long-term sequelae of dental fluorosis in adults are completely lacking. To gain information about this important public health issue, the NIDR contracted with the University of Michigan to compare the oral health of lifetime adult residents of an area with severe endemic dental fluorosis with that of residents in an area with optimally fluoridated drinking water. Cross-sectional examinations were conducted by the Michigan examiners in July 1984. Several variables of oral health including, dental fluorosis, dental caries, tooth loss and periodontal disease were assessed. Findings of the two communities will be compared.

Annual Report of the Field Studies Section
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Field Studies Section was established during the year to conduct epidemiological studies on the broad range of oral disease. One function of this Section will be to collect data on the distribution and prevalence of oral disease and to monitor changes over time. A major effort in this regard has been initiated. In collaboration with the Biometry Section, a contract entitled "Epidemiologic Survey of Oral Health in Adults" was awarded on July 1, 1984. The primary purpose of the adult survey is to establish the prevalence of coronal caries, root surface caries and periodontal destruction in the adult population of the U.S. This survey will establish a baseline to permit future detection of changes and will provide estimates of current treatment needs of employed adults and non-institutionalized retired seniors. After completion of all calibration procedures and pilot studies, examination of the study population will begin by February 1985. The examination phase is expected to run for 12-16 months and will be conducted in the seven geographic regions utilized in the survey of dental caries prevalence in U.S. children, which was completed in 1980.

Another project has been initiated to investigate the prevalence of horizontal bone loss and its utility as a predictor of susceptibility to chronic periodontitis in an adolescent population of American Indians. Chronic adult periodontitis may frequently have its onset in adolescence but is rarely diagnosed until much later. If, indeed, significant numbers of teenagers do exhibit early signs of periodontitis and a simple, effective screening procedure could be devised, an important new approach to the diagnosis and treatment of periodontal disease would be available. Preliminary preparations for this study have been completed and data collection is scheduled to begin in October 1984.

A study to compare the anticaries effectiveness of two NaF/MFP dentifrices at fluoride concentrations of 1000 and 2500 ppm with a control MFP dentifrice of 1000 ppm F is being conducted under contract with the State University of New York at Stony Brook. This study began in 1981 and will terminate in 1985. The first year findings showed no differences between the three dentifrices. Second year data have not yet been analyzed; third year final examinations will be conducted in the spring of 1985.

To investigate the efficacy of sodium fluoride mouthrinsing in preventing dental caries in adults, a study was initiated in 1983 under a contract with the State University of New York at Stony Brook. This three-year clinical study of 1200 adults is being conducted under double blind conditions. Because of initial delays in assembling the study sample, the first year follow-up examination has not been completed at this time and no results are available to report.

Another study conducted by the Eastman Dental Center is designed to determine if the effectiveness of postnatal fluoride supplementation can be enhanced by prenatal administration of fluoride. A total of 1200 pregnant women and their subsequent offspring will participate in the study. At this time approximately 500 mothers have been enrolled. The children of the expectant mothers are to be examined for dental caries at 3 1/2 and again at five years of age; thus the study will not be completed until 1991.

A contract with Colgate-Palmolive Company examining the relationship between tooth fluoride and dental caries was completed during the year. It was concluded that, although fluoride levels in enamel are related to caries, numerous other factors are involved which mask the relationship. Thus, enamel fluoride is not a useful clinical predictor of caries rates. Removal of the outer enamel layer to eliminate the effect of local fluoride did not improve the correlation.

Other studies of periodontal disease are now in the active planning stage, including one concerning chronic periodontitis and tooth loss in adult twins. The Medical Follow-up Agency of the National Academy of Sciences-National Research Council maintains a register of World War II veteran twins. Potential twin samples are presently being compiled for several metropolitan centers in which adequate dental facilities exist.

INTRAMURAL RESEARCH

ANNUAL REPORT OF THE SCIENTIFIC SYSTEMS SECTION

October 1, 1984 - September 30, 1984

The Scientific Systems Section has continued this year to provide data processing support and consulting services to the NIDR Intramural Research Program by operating a central computing facility for the use of both our Intramural scientists and our Administrative Officer, and by providing support for the dedicated laboratory systems in our Institute. The work of this Section proceeded smoothly this year, despite the shortage of personnel caused by the resignations of both a programmer and an electronic engineering technician early in the year, and the lack of secretarial support for a few months mid-year. The programmer has been replaced, and the electronic engineering technician position will be filled shortly with a programmer also.

The central system, although rapidly approaching "old age" for a computer system, has functioned well this year, and provided a high degree of availability for its users. New memory was installed on the system in late spring, providing for a total of 2M Bytes of memory now. The extra capacity enables us to provide better response time to all users, but most importantly to those scientists using the newly installed version of RS/1, the interactive graphics, statistics, and data entry package, which has become increasingly popular this year. Two surplussed large capacity disk drives were acquired and installed this year; one older, lower capacity drive was then removed from the system. The added capacity will help to satisfy the increasing demand for long-term storage on our system. The extra drive will also ease recent difficulties in system backup procedures and allow greater system availability for our users, and flexibility for the programming support staff as well.

Several researchers this year have embraced the workstation concept, and have purchased either a personal computer, if needed for laboratory data acquisition, or a terminal/personal printer combination. Either configuration, when hardwired or connected via a phone line allows access to the central system for data storage, analysis, word processing, recordkeeping, and access via the 11/70 to the DEC10 computer for programming, or file transfer purposes.

A series of programs written originally for a Data General Eclipse S/200 computer system by the Medical Image Processing Group at the Department of Radiology, University of Pennsylvania, is being converted to run on the Diagnostic Systems Branch's VAX 11/750. When completely converted, the software will take digital data produced by CT scans of internal organs in the human body and produce on the DeAnza Image Processor a 3-D reconstruction of a particular site. Its output also includes volume measurements of selected internal structures. Much of the conversion process has been performed on the central system, prior to the arrival of the VAX, so as not to interfere with the normal functioning of the Diagnostic Systems Branch. The software will of course be moved to the VAX for interface to the DeAnza.

Modification to the Neurobiology and Anesthesiology Branch's neurophysiology laboratory's analysis software were made this year. The behavior analysis software was modified to include analysis of the newly developed paradigms in use now on the laboratory systems. Major enhancements to the off-line graphics software provided the following new features: maximum neural frequency within the specified time range may be shown, average latency of

selected trials can be calculated and displayed, and the time lapse between the maximum neural frequency and a specific behavior event may be displayed. Several new commands were added to the already extensive current collection.

A new project was undertaken for the ACRF Pain Clinic. Templates were created using C-CALC Spreadsheet Software to enable the maintenance of a daily appointment schedule and a weekly surgery schedule on the computer. Schedules may be printed locally in the Pain Clinic using a remote printer there.

An internal project was undertaken for the Scientific Systems Section in which a user data base was created and an automated method was developed that will enable us, once fully operational, to easily generate disk storage reports for each user on a monthly basis. This gentle reminder to the users, as to the amount and nature of the storage being used, will hopefully result in a more appropriate usage of our facilities with old, no longer needed, files being deleted promptly, thereby allowing us to provide better service to all users.

Several programs on the 11/70 system have been modified to provide users with expanded capabilities. The budget software for both the Mineralized Tissue Research Branch and the Laboratory of Developmental Biology and Anomalies has been enhanced and also modified to operate more efficiently. The DNA program series used by the Laboratory of Oral Medicine has been updated, and statistical analysis has been performed on data from the Laboratory of Developmental Biology and Anomalies. Radioimmunoassay software analysis of data produced by a gamma counter was added to the 11/70 system. This software performs linear regression on control samples, and then analysis of unknown samples.

A new, greatly enhanced version of RS/1, the interactive data analysis package, was installed on the system, thus providing new capabilities and faster performance to the many users of that system. Also updated this year were BMDP, the statistical analysis package, SATURN Word Processing Software, and C-CALC Spreadsheet Software, as well as our operating systems, RSX-11M and RSX-11M+.

Support for the laboratory computers in the Institute has continued this year, with significant effort placed on the replacement of the Diagnostic Systems Branch's 11/34 computer system by a VAX 11/750 system which will provide much greater speed of operation and convenience in performing picture manipulation and analysis. In preparation for the arrival of this system, files were moved via magnetic tape from the 11/34 to the 11/70 and placed on numerous disk packs there for safekeeping. The system installation was delayed several months because of lack of availability from the manufacturer of a disk drive which was part of the original configuration. In early spring a fixed, high capacity drive was substituted at no increase in cost to the Diagnostic Systems Branch, and the system delivery occurred in early summer. The conversion has now successfully taken place, and the system has proven to be very easy to use, and quite reliable. Addition of a floating point processor shortly will greatly improve the overall speed of analysis also.

Both hardware and software enhancements have occurred on the Neurobiology and Anesthesiology Branch's neurophysiology laboratory system this year. In the area of software changes, new paradigms have been added to the many already available, thus permitting great variability in the types of training sessions and experimental runs which may be done. In addition, the newest version of

the RSX-11M operating system has been installed. The installation of the VAX system for the Diagnostic Systems Branch has resulted in the movement of the replaced 11/34 to the Neurobiology and Anesthesiology Branch. This second system has now become fully operational, and allows researchers to have complete access to the real-time behavior and graphics software from both neurophysiology labs. In order to implement a third laboratory and to begin the process of upgrading the present 11/34 systems to newer, more compact, and more easily maintainable hardware, another computer, a powerful software compatible microcomputer data acquisition system, has been ordered for the Neurobiology and Anesthesiology Branch neurophysiologists.

Two new program packages have been developed and released for usage by the Neurobiology and Anesthesiology Branch Pain Section. They were designed to control the timing and sequencing of heat and toothpulp stimuli to patients to determine pain staircase thresholds using dynamically changing stimulation increments. Software on the PRO-350 has been augmented by the addition of a fourth psychological test, the McGill questionnaire. The DEC PRO/Tool kit will shortly be installed on the system, as will a Fortran compiler and color graphics software. The addition, hopefully this fall, of a larger fixed disk drive for the system will make possible enhancements to increase the speed of operation of the several programs now running on the PC.

Finally, due to our limited resources this year, only a few of the normal complement of courses for the user community were offered, including EDT, the new text editor, the BASIC programming language, and RS/1. Continuous tutoring on several topics including publication quality graphs and posters, proceeded as usual.

Our plans for the immediate future include offering a wide range of classes to our user community, performing an assessment of user needs, and formulating, in conjunction with the Computer Services Committee, a plan for automating the local collection of data from scientific instruments in the laboratory, with the ultimate goal of transferring data collected to the central system for storage and analysis.

Mineralized Tissue Research Branch Summary Report

This year marked the inauguration of the Mineralized Tissue Research Branch as a fully functional entity. This is the only NIH Branch/Laboratory devoted to basic and applied research on the skeletal and dental system. The excellence of the investigators in the Branch is well-known, and they have been responsible for many exciting breakthroughs in the bone and cartilage field. In general, the research conducted in the Branch addresses basic questions of structure and function for the complex tissue systems under study, but recently, these have led to rapid application of the results obtained to bone and joint pathology. The Branch covers disciplines ranging from highly sophisticated and mathematically complex biophysical studies to modern cellular biochemistry and molecular biology. All elements of mineralized tissues are studied, from the mineral phase to the cells and matrices forming each composite skeletal or dental tissue.

In the latter part of this fiscal year, the crystal chemistry portion of the Skeletal Biophysics Section (including the Section Chief, Dr. E.D. Eanes) moved to occupy new laboratory facilities at the National Bureau of Standards, where it will represent NIDR in an outstanding consortium of physical and material scientists working on dental problems. The biological input of the MTRB scientists is a major strength of this grouping. Accompanying these administrative shifts, some delays in research activity are anticipated while the new laboratory facilities at the NBS and the NIDR undergo renovation. However, these delays will hopefully be of short duration, and the new facilities should lead to enhanced productivity for all concerned.

Skeletal Matrix Biochemistry Section

Prior work from the Skeletal Matrix Biochemistry Section established that certain noncollagenous matrix proteins in bones and teeth are either specific gene products of these tissues or, at the very least, quite highly enriched in them compared to other connective tissue systems. These proteins are biosynthetic products of the cells forming the mineralized tissues and probably determine most of their unique biological functions. The proteins are intensely modulated in acquired and heritable disorders of the skeletal system and provide excellent markers for assessment of mineralized tissue pathologies. Study of these proteins is organized in the Section along three interdependent, disciplinary lines of investigation; protein chemistry and immunochemistry, cell biology and molecular biology.

In the past year, it was found that normal bone of some eight species contained similar noncollagenous protein distributions. Osteonectin, the bone proteoglycan and the bone sialoprotein were purified from human bone and antisera raised to the human osteonectin. These antisera were used to set up a radioimmunoassay (RIA) to detect circulating levels of osteonectin in serum. The preliminary results indicate that there is about ten times as much osteonectin in human serum as osteocalcin, the bone gla protein. Further, total osteonectin-like activity in serum increases logarithmically with age. When the RIA is used with HPLC molecular sieve fractions of serum, lower molecular weight forms (probable breakdown products) of osteonectin are detected. These forms are significantly increased in patients suffering from diseases of high bone turnover, such as hyperparathyroidism and Paget's disease. This technique, then, shows high promise for future success in both the diagnosis and assessment of treatment for metabolic bone disease.

Work continued on the assessment of bone matrix defects in osteogenesis imperfecta, the major heritable disorder of bone. In the recently described bovine models for this disease, one variant had decreased levels of osteonectin (2% of normal), bone proteoglycan (10% of normal) and dentin phosphophoryn (associated with the concomitant dentinogenesis imperfecta, 10% of normal dentin values). A second bovine variant, also an autosomal dominant with similar clinical features but somewhat less severe osteopenia, had normal levels of bone osteonectin and dentin phosphophoryn, but only about 25% of the expected bone proteoglycan. A recently discovered sheep variant (with no accompanying dentinogenesis imperfecta) had normal osteonectin but decreased bone proteoglycan. Surveys of bone from human patients suffering from this disease revealed a wide spectrum of biochemical abnormalities. Some children had little or no osteonectin and/or bone proteoglycan (like the bovine/ovine models), while others had normal bone-specific proteins, but deranged type I collagens.

A signal achievement in bone cell biology was achieved this past year. While searching for an optimum method for establishing osteoblasts in vitro, it was discovered that in medium with reduced Ca^{+2} content, cells can be grown from small chips of bone that were previously treated with collagenase to remove adherent non-osteoblastic cells. Cells from a variety of species, including cow, sheep and humans (the latter, taken from bone biopsies or bone removed at surgery from individuals ranging in age from pre-natal to senescence) were found to produce alkaline phosphatase, to form increased levels of cAMP in response to parathyroid horomone, and to produce a thick, extracellular matrix that mineralizes in culture. These cells fully express the osteoblastic phenotype in that they produce large amounts of type I collagen without contaminating type III collagen, as well as the bone-specific noncollagenous proteins, osteonectin and the bone proteoglycan. This discovery represents a quantum leap in bone cell culture and isolation by completely eliminating the problem of fibroblastic and other cell contamination, and by its applicability to all forms of trabecular bone, regardless of origin.

This bone cell culture system was applied to the two bovine, the ovine and several human forms of osteogenesis imperfecta bone. In brief, one bovine form of the disease had cells that were fully competent to make osteonectin (while this protein was not found to be present in vivo) but failed to incorporate the bone proteoglycan into their cell layer extracellular matrix space. This pattern was also seen in the ovine form of the disease. Further, in at least one human patient with progressively deforming OI and no demonstrable type I collagen defects, osteonectin and the bone proteoglycan were synthesized, but not incorporated into the extracellular matrix-rich cell layer fraction. Thus, new insights into the molecular variability of this heritable bone disorder are being provided by this line of experimentation.

Work was initiated this past year toward developing cDNA probes for the bone-specific proteins. Translation of total bone cell RNA followed by immunoprecipitation demonstrated that osteonectin is synthesized as a 47,000 M_r species (SDS gels) before cellular processing. Bone cell cultures labeled for 20 minutes, on the other hand, secrete a 38,000 M_r osteonectin molecule. Similarly, the bone proteoglycan core protein is translated as a doublet, 38,000 and 40,000 daltons in size. In twenty minutes of whole cell synthesis, these proteins reach 42,000 and 51,000 daltons as the two cores are

posttranslationally processed. Current work entails the construction of cDNA libraries to the osteoblast for experiments aimed at screening for osteonectin clones using oligonucleotide primers made to complement the osteonectin amino-terminal sequence. Alternatively, we have inserted the bone cell cDNA into the expressing vector, λgt11 in order to identify synthesized osteonectin using immunochemical criteria. Similar strategies will be used to clone the bone proteoglycan gene.

Proteoglycan Chemistry Section

Proteoglycans are ubiquitous components of connective tissues, where they play major roles in determining the physical properties of these tissues, and where they serve a number of biological functions. Proteoglycans have complex structures and differ markedly in size and composition. The research efforts of the Proteoglycan Chemistry Section are directed toward elucidating the structure, biosynthesis, catabolism and functions of proteoglycans in various tissues and their alteration in pathological states.

Studies carried out this year on cartilage proteoglycans have shown that O-linked oligosaccharides and chondroitin sulfate chains are assembled on the core protein rapidly in the Golgi apparatus of the cell. The kinetics of the entry of ^{3}H from ^{3}H-glucose into the xylose linkage sugars showed that the formation of xylosylserine, which initiates chondroitin sulfate synthesis, is a late step, probably occurring concurrently with chain elongation in the Golgi. In other studies, the N-terminal peptide portion of the proteoglycan core protein, obtained in tryptic digests of the proteoglycan, was identified and partially sequenced. It is located in the hyaluronate-binding region of the molecule. However, the N-terminus in newly synthesized proteoglycans is blocked. Monoclonal antibodies against the hyaluronate-binding region have been prepared. The epitope for one of them has been localized to a peptide in the hyaluronate-binding region which contains a cysteine residue. This epitope appears to be highly conserved since it is present in cartilage and aortic proteoglycans with hyaluronate-binding regions from a wide range of species. In other studies, catabolin, a purified polypeptide isolated from synovial tissue, was shown to induce viable chondrocytes to inhibit synthesis and augment turnover of proteoglycans in articular cartilage explants. This effector appears important in altering proteoglycan metabolism in cartilage undergoing inflammatory or arthritic responses.

The structure and catabolism of cell surface proteoglycan have been studied in rat ovarian granulosa cells. Heparan sulfate proteoglycans on the cell surface are either shed into the medium (40%) or internalized (60%) with a half life of 3-4 hours. The internalized proteoglycans are either degraded directly by transport to lysosomes with a half life of about 30 min, or are degraded in a sequential pathway involving: first, proteolysis and limited endoglycosidic digestion perhaps in endosomes; second, further discrete endoglycosidase activity; and third, complete digestion in lysosomes. This second pathway has a half life of about 3 hours, most of which is spent between steps 2 and 3. Other cells, such as hepatocytes have similar intracellular proteoglycan degradation pathways.

Bone Cell Biology Section

The major goal of the research conducted by the Bone Cell Biology Section has been the elucidation of the mechanism of local control of bone cell differentiation by extracellular matrix components. As in prior years, the major model for this process as studied by these investigators is the heterotopic bone induction system from implants of demineralized bone matrix. The major emphasis of the investigations conducted this past year has been on the characterization and partial purification of the constituent bone inductive proteins and on the development of suitable bioassays to evaluate the differentiation of chondrogenic cells initiating the bone formation cascade.

A major effort toward purification of the bone inductive proteins was initiated this year. One kilogram lots of sized, bovine bone particles served as an abundunt source of these materials. The bovine bone proteins were extracted using 4 M guanidine HCl following conventional acid demineralization. Rapid isolation of biologically active protein(s) was effected by: (1) Amicon ultrafiltration followed by direct ethanol precipitation of total extract protein; (2) affinity chromatography on Heparin-Sepharose and then Hydroxyapatite, bioactivity in each case being included in the bound protein fraction; and (3) rapid gel filtration chromatography on HPLC using TSK-3000 columns in 4 M guanidine HCl. This resulted in an active fraction (heterotopic implant assay) for bone induction exhibiting 3 major and a few minor bands (silver-staining criteria) in a molecular size range of 14 to 26 kilodaltons on SDS electrophoretic slab gels. Current work is aimed at further purification of these proteins by reverse phase HPLC chromatography and preparative gel electrophoresis in the hope of identifying which is responsible for the bone inductive activity.

Studies with bone inductive proteins from other species (e.g., porcine bone) showed that comparable molecular weight fractions (less than 30,000) were all osteoinductive in rat implants. Conversely, bone inductive proteins could not be demonstrated in preliminary experiments using extracts of rat femoral bone marrow, chondrosarcomas or osteosarcomas. Thus, the cellular origin of the bone inductive proteins remains obscure at this point in time.

Other studies were conducted to examine the chondrogenic activity of bone inductive proteins in vitro. As shown previously, demineralized bone matrix can transform embryonic muscle mesenchymal cells to form cartilage in explant cultures. This activity was demonstrated to result from the low molecular weight bone matrix fractions described above. In separate experiments, early invasive cells (3 days post-implantation) were isolated as a mixed cell population from heterotopic rat bone induction implants. These cells were then maintained as suspension cultures. Preliminary data suggest that under these conditions of culture, the early implant-derived cells go on to form cartilage matrix in vitro based on indirect immunofluorescence criteria. Further work is being conducted to demonstrate this point via biosynthetic experiments. Such studies should prove useful in examining the sequence of events leading to chondrocyte differentiation along the endochondral bone formation pathway.

Skeletal Biophysics Section

In mid 1984, the crystal chemistry group of the Skeletal Biophysics Section relocated to occupy new laboratory facilities at the National Bureau of Standards in Gaithersburg. At this new locale, the NIDR-MTRB investigators will work closely with other crystal chemists and materials scientists from the Dental Materials Groups of the NBS and the American Dental Association Health Foundation Program, also based at the NBS Gaithersburg facility. This grouping of investigators devoted to the mineral phase of dental and skeletal tissues is unmatched throughout the world. The combining of the NIDR-MTRB biomineralization expertise with the materials science excellence of the NBS scientists offers high promise for future success in this important area of dental research. The matrix nuclear magnetic resonance (nmr) research team of the Skeletal Biophysics Section will remain in their present laboratory facilities at the NIH Campus in Bethesda.

The overall goal of the research conducted by the crystal chemistry group of the Skeletal Biophysics Section is to acquire a more complete understanding of biomineralization mechanisms, with special emphasis on the inorganic nucleation/growth processes which occur during the initial stages of mineral deposition in vertebrate hard tissue. Areas of interest to these investigators include the physical-chemical and ultrastructural properties of calcium phosphate salts and the interaction of these salts with compounds of biological interest.

During the past year, work continued on the study of membrane-controlled precipitation of calcium phosphate salts using multilamellar liposomes as models for the lipid-mediated mechanisms of mineral deposition in cartilage matrix vesicles. These studies, described in their initial stages last year, suggested that transmembrane transport of precipitable electrolytes can initiate a complex chain of inorganic reactions involving several precursor calcium phosphate phases eventually leading to apatite formation. The close association of these solid mineral phases with the liposomes suggests also the possible importance of the vesicle membrane surface as a site for crystal nucleation, growth and proliferation. Future work will focus on utilization of unilamellar liposome preparations to further validate these conclusions.

Other studies conducted by the crystal chemistry group were aimed at the chemical, kinetic and structural properties of the mineral phases present in pathologic as well as physiologic mineral depositions. Dr. John L. Meyer completed a study on the minerals important to urinary lithiasis and in the early part of 1984 left the MTRB to pursue a career with the Division of Research Grants, NIH. Detailed, rigorous chemical and structural analyses of synthetic apatite analogues were performed leading to a better chemical and physical description of these materials. Studies aimed at verifying previous reports implicating dicalcium phosphate dihydrate (DCPD) as a key intermediate in developing bone failed to confirm the presence of this salt in embryonic chick bone mineral fractions. Similarly, separate studies on calcium phosphate precipitation indicated that DCPD is an unlikely intermediate for apatite under conventional physiological solution conditions. Perhaps special metabolic requirements may be necessary to produce this compound in biological tissues.

The major goal of the research conducted by the matrix nmr group of the Skeletal Biophysics Section is to determine the molecular dynamics, structure and interactions of connective tissue macromolecules, especially those of bone and cartilage, and to elucidate their complex, structure-function relationships. In pursuit of these aims, several innovative biophysical and biochemical approaches were devised and specific nmr instrumentation built by these investigators to perform detailed experiments demonstrating the structural dynamics of the major macromolecules of the mineralizing tissues.

In summary, these investigations demonstrated that collagen fibrils in tissues exhibit specific intermolecular interactions at physiological pH that result in restricted motion of the collagen peptide backbone structure. These interactions chemically stabilize the collagen fibril and contribute significantly to its strength and overall mechanical stability. However, the collagen molecule still retains segmental flexibility over a wide range and is therefore able to distribute stress and dissipate applied mechanical energy. In bone, the flexibility of collagen is substantially reduced, almost assuredly as a consequence of specific interactions between collagen and mineral. This affords bone with the unique biomechanical properties necessary for its skeletal support function. In cartilage, however, compressive loads are dissipated via its proteoglycan constituents. The nmr studies of this tissue showed that its resident proteoglycans are extremely flexible, rapidly changing conformation and absorbing applied energy. This molecular compressibility directly correlated with the ionic composition of intact cartilage. Calcium specifically increases macromolecular proteoglycan viscosity, perhaps by providing a proteoglycan-specific intermolecular network at key points in the supramolecular architecture. Future work is planned to determine further the specifics of the macromolecular and matrix-mineral interrelationships of bone and cartilage that result in the unique structural properties of these tissue systems.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00012-22 MTRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fowler, Bruce O.	Research Chemist	NIDR MTRB

COOPERATING UNITS *(if any)*

Dr. S. Kuroda, Tokyo Medical and Dental Univ., Japan; Dr. J. Christoffersen, Univ. of Copenhagen, Denmark

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Skeletal Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.00	1.00	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (e.g., hydroxide, fluoride, chloride, carbonate, water and acid phosphate). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependency and polarization) are then utilized to establish compositional and structural details of the apatites in question which include: the type and geometry of constituent ions; the site or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00074-11 MTRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Bone and Tooth Matrix Biochemistry and Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Fisher, Larry W.	Staff Fellow	NIDR MTRB
Termine, John D.	Chief	NIDR MTRB

COOPERATING UNITS (if any) S.W. Whitson, SIU, School of Dentistry, Edwardsville, IL; S. Weinstein, U. Iowa Med. School; J. Menczel, FIC, NIH; M.A. Drum, CIPC, NIDR; H. Tenenbaum, LDBA, NIDR

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Skeletal Matrix Biochemistry Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER.
3.59	1.34	2.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The extracellular matrix proteins of the bones and teeth are key elements in the structure and metabolism of these tissues. The goal of this project is to study matrix proteins specific to each mineralizing skeletal tissue in order to understand their molecular structure and biological function.

Analytical procedures (polyacrylamide gel electrophoresis, immunoblotting, specific dye-binding, RIA, ELISA, etc.) have been developed to quantitate the levels of bone specific noncollagenous proteins in (a) surgical specimens of bony tissue (osteonectin, bone sialoprotein and bone proteoglycan) and (b) serum (osteonectin). Changes in the noncollagenous protein profile with age and variety of bone (and tooth) diseases have been observed in man and several animal models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00088-11 MTRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical, Structural, and Morphological Studies on Calcium Phosphates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Eanes, E.D.	Chief, Skeletal Biophysics Section	NIDR MTRB
Doi, Y.	Visiting Fellow	NIDR MTRB

COOPERATING UNITS *(if any)* Dr. J.L. Costa, NIMH, ADAMHA; Dr. R.E. Wuthier, University of South Carolina, Columbia, South Carolina

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Skeletal Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.00	1.50	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium phosphate salts provide the hardness and rigidity which uniquely characterize normal, healthy bones and teeth. Developmental defects in the deposition of these salts or their destruction and loss by disease can severely impair the function of these skeletal tissues. The puspose of this project is to study the physical, chemical, and ultrastructural properties of these salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable these salts to carry out their specialized role in vivo. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. Topics under current investigtion include the formation and properties of precipitates induced in phosphate solutions by the ionophoric translocation of calcium ions across lipophilic solvent barriers, and (2) the formation of dicalcium phosphate dihydrate (DCPD) under physiological-like aqueous solution conditions. The ionophore experiments are being conducted with liposomes with the goal of better understanding how matrix vesicles, the loci for initial mineralization in many vertebrate hard tissues, can concentrate to precipitable levels calcium and phosphate in their membrane and interior spaces. The purpose of the DCPD experiments is to better elucidate possible roles for this phase as a mineral component in skeletal tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00134-10 MTRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Structure and Biosynthesis of Proteoglycans

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

Hascall, Vincent C.	Chief, Proteoglycan Chemistry Section	NIDR MTRB
Yanagishita, M.	Visiting Scientist	NIDR MTRB
Thompson, H.	NIH Postdoctoral Fellow	NIDR MTRB
Stevens, J.W.	Arthritis Foundation Fellow	NIDR MTRB
Morales, T.	Kroc Foundation	NIDR MTRB
Oike, Y.	Visiting Fellow	NIDR MTRB
Vosburgh, F.	Staff Fellow	NIDR MTRB

COOPERATING UNITS (*if any*) Dr. J. Hartford, NIH; Dr. J. Kimura, Rush - Presbyterian - St. Lukes Med. Center; Dr. T. Wight, Un. of Washington, Seattle; Dr. S. Lohmander, U. Lund, Sweden; Dr. M. Farquhar, Yale Un.; Dr. B. Caterson, Un. of West VA; Dr. S. Lamberg, Johns Hopkins Hosp.; Dr. J. Dingle, Strangeways Lab., Cambridge, England,

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.25	6.50	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The purpose of the project is to study the chemical and physical properties and biosynthesis of proteoglycans in a number of tissue and cell systems. Topics of present interest include: 1) Protein chemistry and immunology of the hyaluronic acid-binding region of proteoglycans from the Swarm rat chondrosarcoma; 2) Biosynthesis of the core protein precursor, and the completed proteoglycan with its associated oligosaccharides and of the link protein by chondrocytes; 3) Metabolism of proteoglycans by rat ovarian granulosa cells; 4) Effects of bacterial endotoxins (lipopolysaccharides), catabolin and vitamin A on the regulation of proteoglycan metabolism in organ cultures of bovine articular cartilage; 5) Characterization of the keratan sulfate-proteoglycan in cornea; 6) Proteoglycan synthesis by skin epithelial cells.

Too, C.	Visiting Fellow	NIDR MTRB
Handley, C.	Guest Worker	NIDR MTRB
Morris, J.	IPA	NIDR MTRB
Midura, R.	Biologist	NIDR MTRB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00157-09 MTRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biophysical Studies on the Structure of Connective Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Torchia, Dennis A.	Biophysicist	NIDR, IR, MTRB
Hiyama, Yukio	Visiting Associate	NIDR, IR, MTRB
Sarkar, Susanta K.	Visiting Fellow	NIDR, IR, MTRB

COOPERATING UNITS (if any)

Dr. Paul E. Young, York College, SUNY, Jamaica, N.Y.: Dr. John Thompson, NIDR, IR, LMI; Dr. D. L. VanderHart, CMR, National Bureau of Standards, Washington, DC

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Skeletal Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
4.50	2.75	1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to investigate the molecular structure of proteins and proteoglycans, and to study metabolism in intact Streptococcus lactis. The structural and dynamical information obtained will be correlated with function. Areas of present interest are 1) Molecular structure and dynamics of collagen. Carbon-13, nitrogen-15 and deuterium nuclear magnetic resonance techniques are being used to study the molecular dynamics and interactions in collagen fibers and in appropriate model compounds. 2) Proteoglycan dynamics and interactions with cations. Carbon-13, sodium-23, potasium-39 and calcium-43 nmr spectroscopy are being used to study the interaction of glycosaminoglycan chains with cations in cartilage and in solution. 3) Phosphorous-31 nmr techniques are being developed and applied to study in vivo metabolism in Streptococcus lactis. For these studies high sensitivity magnetic resonance spectrometers have been assembled which give multinuclear spectra of solution and solids. High power decoupling, crosspolarization, magic angle spinning, and solid echo experiments are all performed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00162-08 MTRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Kinetic and Thermodynamic Characterization of Calcium Phosphate Precipitation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Meyer, John L.	Research Chemist	NIDR MTRB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Skeletal Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
.50	.50	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this work is to determine the thermodynamic and kinetic factors which regulate the nucleation, crystal growth and maturation of calcium phosphate crystals. This is accomplished by estimating free ionic activities in solution for all species involved in the crystallization process and relating these terms to the observed precipitation steps. A further correlation is then made between the composition of the solution and the properties of the solid calcium phosphate phase in equilibrium with it. The effect of crystallization inhibitors on the precipitation of calcium phosphates is also being studied in order to elucidate their mode of action at crystal surfaces. Emphasis is placed upon inhibitors which occur naturally in physiological systems or which are common therapeutic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00204-07 MTRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Local Control of Bone Development by Extracellular Matrix Components

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Reddi, A.H.	Chief, Bone Cell Biology Section,	NIDR MTRB
Kuberasampath, T.	Visiting Associate	NIDR MTRB
Muthukumaran, N.	Visiting Fellow	NIDR MTRB
Kapur, S.	Guest Researcher	NIDR MTRB
Landesman, R.	Guest Researcher	NIDR MTRB
Chen, G.	Chemist	NIDR MTRB
Ford, D.	Biologist	NIDR MTRB

COOPERATING UNITS (if any) Dr. Ken McCarthy, AFFRI, Bethesda, MD; Dr. Paul Price, Univ. of Calif.,San Diego; Dr. W.H. Knopse, Rush Med. Coll., Chicago; Dr. M. Nathanson, New Jersey Medical School

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

Bone Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
4.80	4.25	1.55

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to investigate local control of bone differentiation by extracellular matrix components. We have established in previous work the experimental model of bone matrix-induced cartilage and bone development. Projects currently under investigation are: 1) Purification and characterization of bone inductive proteins from bovine bone, 2) Comparative aspects of bone inductive proteins with reference to porcine bone, 3) Possible cellular source and origin of bone inductive proteins, 4) Influence of biphosphonates on cartilage and bone development and mineralization and, 5) The nature of responding cells and their phenotypic potential for chondrogenesis and osteogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00379-01 MTRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bone and Tooth Gene Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Young, Marian F.	Staff Fellow	NIDR, IR: MTRB
Termine, John D.	Chief	NIDR, IR: MTRB

COOPERATING UNITS *(if any)* Sodek, MRC Group in Bone Physiology, University of Toronto; H. Shimokawa, Tokyo Medical and Dental University, Tokyo, Japan; M. Sobel, Lab. of Pathology, National Cancer Institute; M. Bolander, Y. Yamada, LDBA, NIDR

LAB/BRANCH
Mineralized Tissue Research Branch

SECTION
Skeletal Matrix Biochemistry Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.58	1.33	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The matrix proteins of bones and teeth play key roles in the structure and function of these tissues. Our objective in this investigation is to study the biosynthesis of these macromolecules and to understand the regulation of their expression.

The expression of bone and enamel matrix proteins has been studied using RNA from bone cell cultures or ameloblasts and cell free translation. Specific matrix translation products have been successfully identified using polyclonal antisera directed towards each matrix protein. Recombinant cDNA libraries are currently being constructed with the goal of isolating individual genes encoding bone and enamel matrix proteins. These genes will be used to determine the primary structure of these proteins as well as the regulation of their expression during development and disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00380-01 MTRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolism of bone cells in vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robey, Pamela Gehron	Sr. Staff Fellow	NIDR-MTRB
Denholm, Laurence J.	Visiting Fellow	NIDR MTRB
Termine, John D.	Chief	NIDR MTRB

COOPERATING UNITS *(if any)* Thomas D. McClain, Surgery Br., NCI; Michael P. Whyte, Metabolic Res. Unit, Shriner's Hosp., St. Louis, MO; Rogely Boyce, Dept. Vet. Pathol., Texas A&M Un., College Station, Texas; M. Anne Drum, CIPC, NIDR

LAB/BRANCH

Mineralized Tissue Research Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.33	1.83	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The establishment of bone cells in vitro that exhibit and maintain an osteoblastic phenotype has long been a goal of the cell biologist interested in bone metabolism in health and in disease. By utilizing a new technique, osteoblasts from a variety of species (human, bovine and ovine) have been utilized to study the biosynthesis of bone-specific, non-collagenous extracellular matrix proteins, and to examine their potential physiological role and possible alterations in disease states. These cells also serve as a source of mRNA and DNA for studies of non-collagenous, bone specific proteins at the genomic level.

In addition, the culture of tissue from various portions of the tooth is being used to establish cell populations including odontoblasts in vitro to study the biosynthesis of proteins (including phosphophoryn) involved with the formation of this hard tissue.

LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY
Summary Statement
October 1, 1983 - September 30, 1984

During the past year several investigators and technical support personnel from the National Caries Program were transferred to the Laboratory of Microbiology and Immunology. This presented us with some problems because of the limitations in our physical facilities and constraints on our budget for equipment and supplies. However, it is a credit to our senior staff who expended considerable time and effort to coordinate this alignment and to integrate the personnel involved and their research interests into the overall programs of this Laboratory.

Last year also saw a change in leadership in our Cellular Immunology Section. Dr. Sharon M. Wahl was appointed Section Chief. She replaces Dr. Joost J. Oppenheim who became a Branch Chief in the National Cancer Institute. Considerable time was also spent in recruiting personnel to give an adequate manpower base to the Humoral Immunity Section which emphasizes pathogenic mechanisms involved in infectious diseases of the oral cavity.

As in previous years, our accomplishments are summarized according to section activities.

The *Microbiology Section* continues its broadly based research program in ecology, biochemistry and molecular biology. The program seeks to explain physiological and pathological traits of oral microorganisms in molecular terms. The underlying theme of this program is founded on the premise that new knowledge accruing from fundamental inquiries will establish a basis for the formulation of rational approaches to the control or eventual eradication of oral diseases having a microbial etiology.

The ecology program has expanded its investigations on intergeneric coaggregation reactions that occur among various members of the oral microbial flora and which appear to be of major importance in the development of dental plaque. Our previous studies dealt with attempts to delineate the different types of coaggregation reactions that occur between several pairs of oral bacteria. This work led to the formulation of testable models that explained the specificity and diversity of these cell-to-cell interactions. The models make certain predictions about the intergeneric cell recognition systems that extend beyond interacting pairs of bacteria. For example, *Bacteroides loescheii* is known to coaggregate with both *Streptococcus sanguis* and *Actinomyces israelii*. *S. sanguis*, however, will not coaggregate with *A. israelii*. The model for this system predicts, therefore, that a tripartite intergeneric coaggregation complex should be formed if the three organisms are mixed together. This has been demonstrated to occur. Model predictions have now been used to select oral organisms representing six different genera that form hexapartite coaggregation complexes. Results from these and related studies not only reinforce the validity of the proposed coaggregation models, but also provide a means for forming specific mixed microbial complexes *in vitro* that may simulate dental plaque.

Significant progress has also been made in our efforts to isolate and chemically characterize cell surface components that mediate certain of the coaggregation reactions. Several strategies have been employed to investigate

this difficult problem. One involves the use of recombinant DNA technology and is detailed later under our molecular biology program. Another approach involves the use of bacteriophage. Last year we reported the isolation of a virulent phage for *A. viscosus*. It was shown that mutants of *A. viscosus* that had lost the capacity to bind the phage had also lost the ability to participate in a lactose-insensitive coaggregation reaction with *S. sanguis*. Thus, the phage appeared to offer a useful tool for characterizing the *A. viscosus* cell surface component involved in this coaggregation reaction. We have now isolated six new *A. viscosus* bacteriophage from human plaque and demonstrated that they are different than the original phage which was isolated from sewage. We are currently exploring the possibility that the new phage can be used to identify other *A. viscosus* surface receptors that mediate different types of coaggregation reactions. A third approach to the characterization of coaggregation surface structures involves the use of conventional biochemical techniques. We have found that a coaggregation reaction betwen *A. israelii* and *Cytophaga* sp. is mediated by a lectin-like protein that is localized in the outer membrane of the oral cytophaga. Coaggregation defective mutants of the cytophaga have been shown to lack two outer membrane proteins that are present in the wild type strain. Additional work now in progress is directed toward determining whether one or both of these proteins represent the putative coaggregation lectin.

Our biochemical studies have focused on three aspects of microbial metabolism: 1) mechanisms of carbohydrate and pentitol transport 2) mechanisms of protein turnover, and 3) anaerobic amino acid metabolism. We previously established that the vectorial transport of most fermentable sugars in the lactic acid bacteria is coupled to the transfer of the phosphoryl moiety of phosphoenolpyruvate to the sugar substrate by a complement of enzymes comprising specific sugar: phosphotransferase systems. An elusive mechanistic question concerning these systems is whether sugar transport is accomplished in a single concerted phosphorylation-translocation step, or whether the sugar is first internalized by a non-phosphorylation step and then trapped inside the cell by a subsequent phosphorylation reaction. This question has been addressed in *Streptococcus lactis* using mutants that are defective in mannose (glucose):phosphotransferase (man:PTS$^-$), ATP-dependent glucokinase (GK$^-$), or both (man:PTS$^-$ GK$^-$) and a newly synthesized analogue of glucose, β-O-D-galactopyranosyl-(1-4)-2'-deoxy-D-glucopyranose (gal-2DG). The analogue is transported into *S. lactis* *via* the lactose: phosphotransferase system and appears intracellularly as gal-6P-2DG. The phosphorylated derivative is then cleaved by phospho-β-galactosidase into gal-6-P and 2DG. In the wild type strain, 2DG is also phosphorylated to 2DG-6-P although it is not further metabolized. However, none of the mutants were able to catalyze the phosphorylation of intracellular 2DG. Although this work does not conclusively prove the two step hypothesis for sugar transport in *S. lactis*, it does for the first time demonstrate definitively that the man:PTS can function to phosphorylate a sugar intracellularly.

The work on pentitol transport in *Lactobacillus casei* has also yielded interesting results. Certain strains of *L. casei* are unable to grow on xylitol, but if xylitol is added to a culture growing on ribitol, growth inhibition is observed. This has now been explained by the finding that ribitol grown cells are induced for a xylitol:PTS. Thus, xylitol is transported into the cell as xylitol-5-P which cannot be further metabolized. Instead, it is dephosphorylated by a membrane bound phosphatase and the free xylitol is rapidly exported from the cell. This series of reactions make up a futile cycle which results in the net hydrolysis of ATP and inhibition of cell growth. This represents the second

futile cycle discovered in the lactic acid bacteria. Last year, we reported a similar cycle that operated in S. lactis with the glucose analogue, 2-deoxyglucose, and explained the bacteriostatic action of that compound toward S. lactis.

Another facet of our investigations into mechanisms of regulation of sugar catabolism has also yielded important new information. In collaboration with the Mineralized Tissue Research Branch, NIDR, ^{31}P-NMR spectroscopy has been used to measure steady state levels of glycolytic intermediates and inorganic phosphate in physiologically intact cells of S. lactis. This work has established that pyruvate kinase is a key regulatory enzyme in this organism that can control the amount of phosphoenolpyruvate available for sugar transport on the one hand, or that available for ATP synthesis on the other hand. This regulatory role of pyruvate kinase was predicted from prior in vitro studies and has now been confirmed in vivo using this new technique. This represents the first application of ^{31}P-NMR spectroscopy to the study of metabolic intermediates in the lactic acid bacteria and offers the opportunity to test other regulatory hypotheses formulated from in vitro experiments in whole cells.

We are continuing our work on protein turnover in Streptococcus salivarius. It was established previously that a cell-associated fructosyltransferase (FT) produced by this organism is rapidly inactivated and proteolyzed under certain physiological conditions. We have recently found that the initial inactivation step, which serves to 'mark' the enzyme for subsequent proteolysis, is catalyzed by an NADH oxidase system. Our results indicate that this system generates one or more oxygen radicals which interact with the FT to render it catalytically inactive. Several oxygen radical scavengers (including superoxide dismutase) can prevent inactivation in permeablized cell suspensions.

A new project initiated this year involves an investigation of the anaerobic fermentation of amino acids by oral peptostreptococci. It has been found that this group of organisms ferment various amino acids by coupled oxidation-reduction reaction sequences (Stickland reactions). The amino acid favored as a terminal electron acceptor in these fermentations is proline. This is considered highly significant in the adaptation of the peptostreptocci to the oral environment because of the abundance of proline which is available in the salivary proteins.

The Section's molecular biology program continues to make important contributions in two areas. The first represents a continuing, long range project designed to develop genetic exchange systems for the oral lactobacilli. The second involves the use of recombinant DNA technology to identify and characterize surface molecules on oral bacteria that mediate specific adherence reactions.

With respect to the first area, methods have now been developed for the efficient production and regeneration of L. casei protoplasts. This was a difficult and crucial first step in the development of a genetic exchange system for this group of organisms. In addition, small cryptic plasmids previously isolated from L. casei have been used to construct cloning shuttle vectors. It has now been demonstrated that the protoplasts can be transformed to erythromycin resistance by the newly constructed vectors. The protoplasts have also been shown to be amenable to a liposome-mediated transfection with L. casei

bacteriophage. Technology, therefore, is now available for the transfer of genetic information among the oral lactobacilli.

Last year we reported that a collaborative study with the Humoral Immunity Section resulted in the successful cloning of an *A. viscosus* gene in *E. coli* that expressed a subunit (or precursor) of *A. viscosus* type 2 fimbriae. This fimbrial structure carries the lectin responsible for mediating a highly specific and lactose reversible coaggregation reaction with *Streptococcus sanguis*. We now report that the *A. viscosus* gene has been subcloned on a 4.3 Kbp Bam fragment and this DNA fragment will be sequenced to determine the primary amino acid structure of the protein product.

The research objectives of the *Humoral Immunity Section*, which was restructured a year and a half ago, are currently focused on the pathogenic mechanisms involved in microbial adherence and the host's response to oral microorganisms. Investigation of the recognition processes which initiate the attachment of oral bacteria to other bacteria and to the tooth surface are being continued in an effort to delineate the events involved in the formation and accumulation of dental plaque. Also continuing are studies of the interaction of a major host mediator system, the complement system, with bacteria implicated in the etiology of periodontal disease. During the past year the perspective of this Section has been broadened to include examination of the interaction of bacteria with oral epithelial cells. In addition, studies have been initiated to extend the area of bacterial initiated host responses to the exploration of the effects of IgA proteases on specific IgA mediated interactions with oral bacteria.

Having previously identified two antigenically and functionally distinct types of fimbriae on *Actinomyces viscosus*, major emphasis has been placed on the characterization of the receptor on *Streptococcus sanguis* 34 with which one of these fimbriae (type 2) reacts. The adherence of *A. viscosus* T14V and *S. sanguis* 34 is clearly attributable to a low affinity lectin activity associated with the type 2 fimbriae and a carbohydrate receptor on *S. sanguis* 34. Thus, galactose β(1→3)N-acetyl-galactosamine was found to be a potent inhibitor of adherence as were β-methylgalactoside and lactose although these latter saccharides were less active. Plant lectins specific for galactose β(1→3)N-acetyl-galactosamine, galactose or N-acetylgalactosamine, were found to exhibit a striking similarity to the actinomyces lectin in the agglutination of a large number of streptococcal strains. Based on this finding, the lectin from *Ricinus communis* (RCA_1) was utilized for the negative selection of a mutant of *S. sanguis* 34 which failed to coaggregate with *A. viscosus* T14V. The adsorption of antiserum against *S. sanguis* 34 with the mutant provided a reagent which detected a single antigen in a mutanolysin digest of streptococcal cell walls. A streptococcal receptor associated antigen which inhibits lactose sensitive coaggregation of *A. viscosus* T14V with *S. sanguis* 34 has been obtained by specific retention to and elution from an RCA_I affinity column. Initial chemical characterization of the streptococcal receptor indicates that it is an unusual carbohydrate in which oligosaccharide chains are linked directly by phosphodiester bonds. A hexasaccharide with N-acetyl-galactosamine as the terminal nonreducing sugar appears likely to be the specific lectin-reactive saccharide.

Significant progress has been made in cloning of the genes coding for the actinomyces type 2 fimbriae in *E. coli*. Characterization of the type 2 fimbriae

has been difficult since these structures have not been susceptible to conventional methods of dissociation. An approach for the examination of the subunit structure of these fimbriae which appears to be especially promising is the utilization of cloning techniques. In collaboration with the Microbiology Section, a clone has been found that produces a 60,000 dalton protein detected by monospecific and monoclonal antibodies against the type 2 fimbriae. This protein is similar in size and antigenicity to a protein present in *A. viscosus* cells and to one which has been detected in small amounts in preparations of affinity purified type 2 fimbriae. The presence of a second subunit has recently been suggested by the finding of a protein of approximately 35,000 daltons which reacts with monospecific antibodies against the type 2 fimbriae.

The *in vivo* attachment of *S. sanguis* 34 to *A. viscosus* T14V has been largely inferred from *in vitro* studies of coaggregation. The *in vivo* occurrence of these interactions is strongly suggested by the recent demonstration of the similar distribution of *A. viscosus* T14V type 2 fimbriae and the *S. sanguis* 34 receptor in plaque samples by double labelled immunofluorescent techniques.

The type 2 fimbriae have also been shown to mediate the attachment of actinomyces to epithelial cells. *A. naeslundii* WVU45 (which possesses only type 2 fimbriae) adhered to human oral epithelial cell lines. Neuraminidase treatment of the epithelial cells enhanced attachment by two to three fold, presumably by exposing specific membrane associated carbohydrates. A mutant obtained by its inability to be agglutinated by an antiserum against the type 2 fimbriae adhered minimally. Although the adherence of *A. viscosus* T14V was approximately one-half that of *A. naeslundii*, this process was also mediated by the type 2 fimbriae. The parent strain and a mutant lacking only the type 1 fimbriae attached to the epithelial cells whereas little binding of the mutants lacking the type 2 fimbriae or both fimbriae was detected. Additional support for the interaction of the *A. naeslundii* type 2 fimbriae with the epithelial cells was provided by the finding that Fab fragments of antibodies reactive with the type 2 fimbriae inhibited attachment. The lectin-carbohydrate nature of this interaction was supported by the finding that attachment of *A. naeslundii* was inhibited by lactose and β-methylgalactoside but not by α-methylgalactoside or monosaccharides other than galactose.

The epithelial cell receptor for the actinomyces lectin has been further characterized by the utilization of a variety of plant and invertebrate lectins of known specificity. Lectins specific for galactose β(1→3) N-acetylgalactosamine, N-acetylgalactosamine, or galactose were the most effective inhibitors of the attachment of *A. naeslundii* to oral epithelial cells. Lectins that recognize α-linked galactose or galactose-β(1→4) N-acetylglucosamine were not inhibitory suggesting that the β-anomer of the galactoside is present in the receptor and that the saccharide in the penultimate position participates in the bacterial lectin combining site. It is anticipated that these plant lectins will prove to be extremely useful in the purification and further characterization of the epithelial cell receptors for the actinomyces and possibly other bacteria.

The demonstration of the lectin activity associated with the type 2 fimbriae and the mediation of the attachment of *A. viscosus* to saliva treated hydroxyapatite by the type 1 fimbriae have suggested that the distribution of bacteria within the oral cavity may reflect the presence of fimbriae with specific antigenic and functional properties. Utilizing immunofluorescent techniques, it has been shown that the type 2 fimbriae of *A. naeslundii* are

preferentially found in association with epithelial surfaces. Conversely, the type 1 and type 2 fimbriae of A. viscosus are present in high numbers in dental plaque samples where only minimal fluorescent staining of the type 2 fimbriae of A. naeslundii was observed.

Investigations are continuing to examine the interaction of the complement system with the actinomyces. These initial studies are designed to provide structural information concerning the relative localization of epitopes on the fimbriae of A. viscosus T14V utilizing complement fixation by several monoclonal antibodies reactive with each of the two types of fimbriae. Competetive inhibition of binding of each monoclonal antibody by all the others specific for one fimbrial type is being examined in conjunction with the complement studies. These binding studies, however, identify only the antibodies which react with the same epitope. Complement fixation assays extend these findings to identification of antibodies which bind to specific but different epitopes in sufficiently close proximity to bind and activate the Clq subcomponent of the first complement component. If the two antibodies do not bind to epitopes within a certain spatial distance, Clq is not activated. Recent studies suggest that the epitope repetition sequence of the type 1 fimbriae varies from that of the type 2 fimbriae. Thus, relatively high concentrations of three monoclonal antibodies reactive with the type 1 fimbriae all activate the complement sequence in the absence of an added second antibody. In marked contrast, only one of four anti-type 2 fimbrial antibodies of isotypes which would be expected to activate complement actually do so. However, with the exception of one pair of antibodies, both participants of which interact with epitopes in very close proximity to each other, the addition of one antibody to a second antibody results in the formation of a complement fixing doublet. Several pairs of antibodies reactive with the type 1 fimbriae also exhibit this synergistic effect at concentrations of each antibody which do not activate complement alone. Since the Fc fragments of antibodies interact with Clq, they have been removed from each of the monoclonal antibodies by pepsin digestion. The resulting $F(ab')_2$ fragments in conjunction with an intact antibody of different specificity did not activate complement indicating the participation of the Fc fragments of both antibodies in this activation process. An alternative interpretation of these data is that binding of one monoclonal antibody to its respective epitope significantly enhances the binding affinity of a second antibody specific for a different epitope, an effect which could enhance complement consumption. This seems unlikely since enhanced binding of one antibody initiated by the addition of a second antibody was observed in only one of eleven pairs of antibodies which exhibited synergistic complement fixation patterns.

Studies have been initiated to examine another aspect of the host's response to oral microorganisms; namely the ability of secretory IgA to inhibit attachment of oral bacteria to other bacteria and epithelial cells and the effects of cleavage of the IgA by IgA proteases on these activities. Although the cleavage of myeloma IgAs by specific IgA proteases from several microorganisms has been well documented, virtually nothing is known concerning the biological activities of the resulting fragments. Colostrum obtained from primates (Macaca fascicularis) immunized with actinomyces will provide the source of specific IgA. Since previous investigations indicate a strict species homology between the sources of the bacterial isolates producing the proteases and the IgA, the microorganims are being obtained from the supragingival plaque of primates. Fourteen isolates of bacteria resembling S. sanguis have been obtained. These bacteria have been characterized by several physiological criteria including

enzyme activity, fermentation of sugar and the production of glucan from sucrose. Fluorescent antibodies have identified all but one of these isolates as S. sanguis biotype 1. These bacteria are currently being examined for the production of IgA protease.

The Cellular Immunology Section is exploring the regulation of inflammatory cell recruitment, proliferation, differentiation and activation through the release of soluble faciliatory and/or inhibitory mediators by lymphocytes and monocytes. Host defense to various microbial, neoplastic and other foreign antigens is the consequence of the interaction of a network of immune cells. This interaction is dependent upon the production and release of lymphokines and monokines which provide the molecular information link between these different populations. Our in vitro biological and biochemical analysis of these immunomodulators has been greatly enhanced by the establishment of several important collaborations with investigators in clinical branches at NIH and elsewhere. In situ inflammatory lesions provide a unique opportunity for dissecting the multiple interlocking events in the inflammatory response.

Significant advances have been made in the characterization of one of these human monocyte-derived mediators, Interleukin 1 (IL 1). This hormone like mediator which can act on targets as disperse as lymphocytes, chondrocytes, fibroblasts, hepatocytes and brain, as well as on other macrophages, has now been purified to homogeneity. Purification of this mediator with retention of biologic activity offers the opportunity to explicitly define IL 1 target cells and the precise mechanism of action of this important immunomodulator. In addition, using purified IL 1, monoclonal antibodies can be produced to further define the role of this mediator in the regulation of different cell types participating in inflammation. Molecular cloning and purification of this and other mediators may permit the therapeutic application of these molecules and/or their antibodies to control specific steps in host defense and inflammation. Definition of a role for IL 1 in the degradation of cartilage matrices has been one of the consequences of the availability of this purified mediator. IL 1 was found to directly induce the release of glycosaminoglycans from bovine cartilage explants implicating a role for monocytes and IL 1 in the regulation of cartilage matrix degradation in inflammatory and degenerative joint diseases.

Further characterization of the contribution of monocytes to connective tissue destruction comes from studies demonstrating collagenase production by human peripheral blood monocytes. Although human monocytes participate in immune and inflammatory reactions in which tissue remodeling is known to occur, little is known concerning their direct influence on collagen turnover. While previous studies have demonstrated a role for monocytes in influencing other cells to produce this enzyme (via IL 1), this is the first description of the actual production of collagenase by human monocytes. These exciting observations indicate that human monocytes have the capacity to modulate collagen degradation directly by production of collagenase. Furthermore, the production of collagenase by monocytes can be inhibited by both steroid and nonsteroidal anti-inflammatory drugs which may provide a basis for the effect of anti-inflammatory agents on the amelioration of inflammation-based tissue injury.

Certain chronic inflammatory diseases such as rheumatoid arthritis and chronic periodontitis are characterized by degradation of connective tissue matrices including bone, collagen and cartilage. Since activated mononuclear leukocytes are present in rheumatoid synovia, it suggests that factors released

from these activated cells play an important role in the proliferation of the synovial tissue, the development of the pannus and the destruction of connective tissue characteristic of this disease. Activated mononuclear cells can be isolated from this inflammatory tissue and have been characterized phenotypically and functionally. One of the lymphokines spontaneously produced by these inflammatory cells is a fibroblast activating factor (FAF) which is being characterized and purified for molecular cloning and monoclonal antibody production. Although a wide spectrum of pathologic changes ranging from minimal inflammatory cell infiltration to tissue with widespread necrosis and overwhelming infiltration with inflammatory cells have been demonstrated, members of the Cellular Immunology Section in collaboration with the Arthritis Branch of NIADDK have recently been able to divide arthritis patients into subgroups with common immunologic, pathologic, histologic and therapeutic characteristics. These data suggest that clinically active rheumatoid arthritis is a heterogeneous disorder with polar subgroups who share common features as is seen in certain other chronic inflammatory diseases. These important observations provide insight into potential pathogenic mechanisms and provide a sound basis for therapeutic intervention.

In related studies with NIADDK, an experimental rat model of polyarthritis has been used to effectively assess the contribution of inflammatory cells to arthritis and tissue destruction. This disease process which is initiated by group A streptococcal cell walls appears to be T cell dependent and can be modulated pharmacologically enabling assessment of various therapeutic modalities on the development and progression of this chronic process. One such anti-inflammatory agent is Cyclosporin A, an inhibitor of T cell function, commonly used in transplant patients. This agent has been found to profoundly inhibit the development of chronic arthritis and joint destruction in this model prompting its application to treatment of human rheumatoid arthritis.

The development and differentiation of mononuclear cells to become effector cells is another important avenue of study in this Section. The influence of the lymphokine, colony stimulating factor (CSF), on the proliferation and differentiation of bone marrow hemopoietic cells has been explored using a CSF dependent cell line. The PT-18 cell line was used to elucidate how CSF regulates the progression of the cells through the cell cycle by acting early in the G_1 phase (first 6 hrs) to drive the cells into a proliferative response. The data demonstrate that the events occurring in the G_1 phase are essential for continuation of proliferation and that interruption of these events can interfere with the maturation of bone marrow stem cells into mature, functional monocyte-macrophages capable of participating in host defense to microbial and other antigens.

The inability of monocyte-macrophages to function as normal effector cells in host defense can have profound consequences in the ability of the host to combat bacteria, viruses, tumor cells and other foreign substances. In collaborative efforts with NIAID and the NIH Clinical Center, the Cellular Immunology Section has become actively involved in the search to identify the basic mechanisms responsible for the immunosuppression which characterizes the acquired immune deficiency syndrome (AIDS). Accumulating evidence suggests that the monocytes of these patients are unable to adequately participate in host defense. Using as a target cell, Giardia lamblia, a frequent pathogen in AIDS patients, we demonstrated that monocytes from AIDS patients exhibit defective killing of this extracellular protozoan. In further studies elucidating the

mechanism of this abnormal monocyte killing, it appears that monocytes from these patients cannot generate the elevated levels of the reactive oxygen metabolites, H_2O_2 and O_2^-, required for killing this and, presumably other, infectious organisms. Furthermore, the monocytes from these patients do not migrate normally towards chemotactic stimuli, impeding their ability to participate in immune and inflammatory events. Further studies demonstrating defective production of mediators such as IL 1 and prostaglandins by AIDS monocytes corroborate the potential contribution of this mononuclear leukocyte population to the immunosuppressed condition of these patients. Analysis of the ability of immunomodulators to reverse these monocyte dysfunctions may provide interesting possibilities for therapeutic intervention in host defense mechanisms.

The Clinical Immunology Section continues to study the release of inflammatory mediators from basophils and mast cells. These experiments attempt to better define the mechanisms involved in cell secretion and the nature of the different mediators that are released from cells. A cloned subline of the rat basophilic leukemia cell has been developed with a number of characteristics useful for *in vitro* studies. The cells divide rapidly, grow well *in vitro* attached to plastic and have surface IgG and IgE receptors similar to normal basophils. The cells contain histamine and serotonin. Although obtained from a tumor and heteroploid in chromosomal number, these cells have many similarities to normal mast cells or basophils. The cells can be activated by crosslinking of the immunoglobulin receptors to release mediators such as histamine, serotonin, prostaglandins and other arachidonic acid products. This is an energy requiring step blocked by metabolic inhibitors. Similar indirect information on release mechanisms has been obtained by the use of inhibitors which are thought to interfere with other cellular functions. However, the ready availability of large numbers of cultured rat basophilic leukemia cells allows direct biochemical recognition of the events which occur in cells following activation. Variants of the rat basophilic leukemia cells have also been obtained which are incapable of histamine release. Biochemical characterization of the defect in these cells is useful for determining the sequence of events induced in cell activation and secretion. These events include receptor crosslinking, proteolytic enzyme activation, phospholipid methylation, increased phosphorylation of membrane lipids, Ca^{2+} influx, phospholipase activation, release of arachidonic acid, protein phosphorylation and finally, secretion of histamine. However, not all of these changes have been observed in all cell types; for example, the cyclic AMP increase is seen in mast cells but not in basophils. It is well recognized that the phosphorylation of cellular proteins modulate many cellular functions. We found in recent experiments that the activation of the basophil is accompanied by the rapid phosphorylation of a 92,000 molecular weight protein in the cell. The degree of phosphorylation of this component correlates with the extent of histamine release. This phosphorylation is rapid and occurs before the release of any mediators from the cell. The phosphorylation requires the presence of Ca^{2+} in the medium; however, it is not the result of Ca^{2+} entry into the cell. Phosphorylation was also studied in variant cloned cell lines which have various defects in cell secretion. Phosphorylation occurred in the absence of histamine or arachidonic acid release and when there was no Ca^{2+} uptake by the cells. Therefore the stimulation of a kinase is an early event in the activation of the rat basophilic leukemia cells. A number of different kinases have been described in cells (e.g., cAMP, Ca^{2+} or phospholipid dependent); the characteristics of the present reaction suggests that there is a Ca^{2+} dependent kinase which is activated during stimulation and its characterization should provide important information on the release of mediators from cells.

Variants of the rat basophilic leukemia cell line have been isolated which have drug resistance markers and also defects at different steps in the mediator release pathway. Somatic cell hybridization techniques allow the correction of defects in the different variants and the establishment of complementation groups of these sublines. When cells from histamine release negative variants are fused to cells which are release positive, the hybrid cell responds to stimuli. Therefore, the lack of release is a recessive characteristic. Cell fusions have also been performed between two non-releasing variants of the rat basophilic leukemia cells. If the two cell lines have defects in completely different steps their fusion would result in reconstitution of the release phenotype. Such experiments have demonstrated at lease five complementation groups in the defective cells. This type of analysis will be more powerful as new variants become available.

Recent experiments have combined the techniques of monoclonal antibody production and selection of somatic cell variants to study the immunoglobulin receptors on the rat basophilic leukemia cell. Hybridoma antibodies have been produced to study histamine release from cells. Four monoclonal IgG_1 antibodies were isolated which inhibit the binding of IgE to rat basophilic leukemia cells. Three of these antibodies bind competitively with IgE; the fourth monoclonal (AA4) inhibits IgE binding but is not inhibited by IgE. The number of molecules of the 3 monoclonal antibodies that bind to the RBL cell surface is similar to the number of IgE receptors on the cells. However, there is 10-fold more of the fourth monoclonal (AA4) bound to the cell surface. The 3 hybridomas induce the release of histamine from both normal mast cells and rat basophilic leukemia cells. AA4 does not release histamine but can modulate the release reaction. The 3 hybridomas bind to the α-component of the IgE receptor whereas AA4 appears to bind to a molecule closely associated with the IgE receptor. From the RBL cells, cloned variant lines have been isolated with reduction in both IgE and binding of the different monoclonal hybridomas (reduction of 90%). The binding of the three monoclonal antibodies paralleled the binding of IgE; however, variant lines have been selected with decreased binding of this fourth monoclonal but relatively normal IgE receptor number. Therefore, this (AA4) epitope varies independently from IgE. These monoclonal antibodies and variant cell lines will be useful for studies of the IgE receptor and cell activation. The capacity of the fourth monoclonal antibody to inhibit release following binding to the cell surface strongly suggests that it is binding to membrane structures which are critical in the release process. The identification of this molecule should further our understanding of cell secretion and the means to regulate inflammatory responses.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE-00007-23 LMI

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)
Studies on the Regulation of Carbohydrate Metabolism in Oral Bacteria

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator*) (*Name, title, laboratory, and institute affiliation*)

Charles L. Wittenberger	Chief, Microbiology Section	LMI, NIDR
Roberta Schoen	Microbiologist	LMI, NIDR
Kazuhiko Abbe	Visiting Fellow	LMI, NIDR
Shoko Abbe	Guest Worker	LMI, NIDR

COOPERATING UNITS (*if any*)

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type Do not exceed the space provided.*)

This investigation focuses on resolving the biochemical reactions involved in the inactivation and proteolytic degradation of a cell-associated fructosyltransferase (FTase) produced by Streptococcus salivarius. We have concentrated on the FTase inactivation step because this reaction appears to "mark" the enzyme for subsequent protiolysis. Using both intact and permeablized cells, we have found that the inactivation step requires NADH, Cu^{++} (specifically), and oxygen. It was further shown that the NADH oxidase system is involved in an oxidative modification of FTase that results in a loss of catalytic activity. One or more species of oxygen radicals appear to be involved in the modification-inactivation step.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00022-17 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparative Physiology of Lactic Acid Bacteria and Other Oral Microbes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jack London	Research Microbiologist	LMI, NIDR
Sally Hausman	Microbiologist	LMI, NIDR
Angelika Kagermeier	Visiting Fellow	LMI, NIDR
Paul Kolenbrander	Microbiologist	LMI, NIDR
John Thompson	Visiting Scientist	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. Roger Celesk, Miles Laboratories, New Haven, Conn.
Dr. Harold Neimark, Downstate Medical Center, SUNY, New York, N.Y.
Dr. Othmar Gabriel, Georgetown University, Washington, D. C.

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.20	2.20	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The transient inhibition by xylitol of strains of L. casei growing at the expense of ribitol appears to be the result of a xylitol-driven futile cycle. Growth studies and resting cell experiments demonstrated that L. casei Cl-16 possesses the ability to (1) import xylitol via a specific phosphotransferase system (PTS), (2) dephosphorylate the transport product xylitol-5-P to xylitol and (3) export free xylitol. These enzymatic activities provide the cell with all of the components required to drive a cycle that pumps xylitol into and out of the cell. Since this mechanism requires PEP, its operation depletes the cells' energy supplies. It is this depletion of the energy reserves that accounts for the inhibition in growth, however, sufficient energy remains to permit maintenance functions. Thus, the inhibition is only bacteriostatic in nature rather than bacteriocidal.

The coaggregation of Cytophaga sp. DR 2001 and Actinomyces israelii appears to be mediated by a lectin-like protein component of the outer membrane of the cytophaga. Analysis by SDS-PAGE electrophoresis, western blot techniques and specifically absorbed antisera indicate that the lectin is an outer membrane protein (OMP) with a M.W. of 155,000 that is basic (anodal) in nature. Antisera are now being prepared against proteins suspected of lectin activity. Efforts to isolate the carbohydrate receptor from A. israelii cell walls using specific plant lectins are also underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00034-16 LMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Histamine Release

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.	Chief, Clinical Immunology	NIDR LMI
Hook, William A.	Research Microbiologist	NIDR LMI
Stracke, Mary L.	Medical Staff Fellow	NIDR LMI
Basciano, Luann K.	Microbiologist	NIDR LMI
Berenstein, Elsa H.	Microbiologist	NIDR LMI
Garcia-Gil, Mercedes	Visiting Fellow	NIDR LMI
Essani, Naeen, A.	Visiting Fellow	NIDR LMI

COOPERATING UNITS *(if any)* NIAMDD, Arthritis & Rheumatism Branch, NIH; NHLBI, Laboooratory of Chemical Pharmacology, NIH (M. Beaven); Walter Reed Army Institute of Research (P. Chiang); NIAID, LCI, NIH; Clinical Center, Clinical Pathology Dept., NIH (J. Donlon)

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
4.35	2.85	1.50

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Histamine release from mast cells and blood basophils is being studied as one of the immunological mechanisms involved in inflammation. It is also a model for cell secretion. Among the histamine releasing agents employed are IgE antibody, the anaphylatoxins, and the Ca^{2+} ionophore A23187. Cultured rat basophilic leukemia cells are used as a model for the studies of the IgE receptor and biochemical changes during cell activation. Large numbers of cells can be obtained for biochemical studies and biochemical variants have been selected which are defective at different sites in the pathway of cell activation and secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00042-14 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Molecular Biological Characterization of Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce M. Chassy	Research Chemist	LMI, NIDR
Emily V. Porter	Chemist	LMI, NIDR
Ja-Lee-Wickner	Visiting Fellow	LMI, NIDR
Eunice Hull	Bio Lab Technician	LMI, NIDR

COOPERATING UNITS *(if any)*

None

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Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This project seeks to use genetic, biochemical and physiological approaches to investigate the oral bacteria. Three specific areas of investigation are: A) the use of molecular biological techniques to characterize surface antigens of oral microbes. B) the characterization of plasmids isolated from oral bacteria and C) the study of the regulation of sugar transport systems. A 'genomic library' of Actinomyces viscosus DNA was prepared in Escherichia coli using the cosmid vector pHC79. The library was screened for expression of A. viscosus antigens using immunological techniques. One clone was found that produced a subunit of the Type 2 fimbriae. Western blot analysis has confirmed that this clone produces a protein indistinguishable from a component of the Type 2 fimbriae. The gene has been further subcloned into pUC13 on a 4.3 Kbp Bam fragment an found to produced a truncate 30 Kalton product. Lactose plasmis isolate from strains of L. casei encode the membrane associate lactose transport system (lac-PTS and the soluble enzyme, phospho-β-galactosiase. The plasmids vary in size an restriction enzyme cutting patterns. Southern blots demonstrate that they represent a series of different plasmis carrying homologous lac genes. The multicopy cryptic plasmids were characterize by restriction endonuclease mapping, cloning into E. coli an minicell analysis. Shuttle vectors capable of replicating in E. coli or S. sanguis an L. casei were constructed. Methods for the efficient production and regeneration of L. casei protoplasts have allowed the introduction of DNA into Lactobacillus strains for the first time via a highly efficient liposome-mediate transfection system (10^5 transfectants/ ug DNA). A hexose 6-phosphate phosphohydrolase was purified an characterize. Lactose analogs were synthesize enzymatically and use to produce high intracellular 2-DG an 2-FG system in studies of the effect of intracellular glucose concentrations and the glucose efflux system. First evidence was obtained for the intracellular phosphorylation of glucose and glucose analogs by the glucose-PTS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00043-14 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological and Genetic Studies on Pathogenic Oral Microorganisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jacob A. Donkersloot	Research Microbiologist	LMI, NIDR
Robert J. Harr	Bio Lab Tech (Micro)	LMI, NIDR
Eunice M. Hull	Bio Lab Tech	LMI, NIDR

COOPERATING UNITS (if any)

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Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.5	1	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have been initiated to isolate and examine the genes encoding type 1 and type 2 fimbriae from Actinomyces viscosus in order to investigate the structural organization and biosynthesis of these fimbriae. Cosmid cloning was used to establish a gene library of A. viscosus T14V in Escherichia coli. Restriction analysis showed that on a statistical basis ($P > 0.99$) this library of 550 clones represented the entire A. viscosus genome. Colony immunoassays showed that 25 clones reacted with antiserum directed against A. viscosus cells. The proteins responsible for these reactions were identified in 3 clones. Clones AV1209 and 2009 expressed non-fimbrial antigens of 40 and 58 kilodalton, respectively. Clone AV1402 expressed a 59 kilodalton protein that reacted with monospecific antiserum against type 2 fimbriae; this protein was identical in size (as judged by electrophoretic mobility) to a component of type 2 fimbriae. Thus, a gene (fim A) encoding a type 2 fimbrial subunit has been isolated and is expressed in E. coli.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE- 00046-13 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Chronic Inflammation and Immunomodulation of Connective Tissue Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Sharon M. Wahl	Chief, Cellular Immunology Section	LMI	NIDR
Kiyoshi Ohura	Visiting Fellow	LMI	NIDR
Maria Agelli	Visiting Fellow	LMI	NIDR
William Helsel	Microbiologist	LMI	NIDR
Denise Hunt	Microbiologist	LMI	NIDR
Sueanne Dougherty	Microbiologist	LMI	NIDR

COOPERATING UNITS (if any)

Dr. R. Wilder, ARB, NIADDK
Dr. D. Yocum, ARB, NIADDK
Dr. K. Foon, NCI
Dr. I. Kotona, NMRI, Navy
Dr. R. Siraganian, Clinical Immunology, LMI,NIDR
Dr. F. Robey, FDA

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Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL.	OTHER
4.8	2.00	2.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Investigations in this laboratory continue to examine the regulatory role of lymphocytes and macrophages in connective tissue metabolism. The influx of mononuclear cells into the synovium in rheumatoid arthritis appears to influence the hyperplasia of synovial fibroblasts which produce excessive levels of the inflammatory mediators, collagenase and prostaglandins. Lymphocytes and macrophages can stimulate this fibroblast growth and regulate fibroblast function via soluble intercellular signals, lymphokines and monokines. The mediators are being characterized, purified and messenger RNA isolated for translation of these mononuclear cell products. Subpopulations of fibroblast target cells which specifically respond to these mediators are being cloned and identified by specific cell surface receptors and by unique functional capabilities. The T cell dependence of the synovial fibroplasia is emphasized by the histopathology of the tissues. Synovial tissues from one subset of arthritis patients (anergic) have intense T cell infiltration, a high ratio of helper:suppressor T cells and many HLA-DR$^+$ cells. These synovia also have more synovial lining cell hyperplasia. In contrast, another subset (nonanergic) of patients have few T cells and also have less fibroplasia. The T cells isolated from inflamed synovium constitutively produce factors which stimulate fibroblast growth. Additional studies indicate that peripheral blood mononuclear cell immune function reflects immunopathologic events in the affected joint. These data provide insight into potential pathogenic mechanisms associated with rheumatoid arthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-DE-00061-08 LMI

PERIOD COVERED

October 1, 1983- September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Complement Activation and Inflammation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ann L. Sandberg, Chief, Humoral Immunity Section, LMI, NIDR
John Cisar, Research Microbiologist, LMI, NIDR
Linda Lewis Mudrick, Microbiologist, LMI, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity Section

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.9	.9	1.

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several aspects of the interaction of the complement system with Actinomyces viscosus T14V, an organism implicated in the pathogenesis of periodontal disease, are under exploration. Complement fixation by monoclonal antibodies is being used to localize epitopes on the fimbriae of these bacteria and associate them with functional properties of the fimbriae. Antibodies with different specificities for the type 1 fimbriae (which mediate attachment to saliva treated hydroxyapatite) and the type 2 fimbriae (which are associated with lectin activity for saccharide receptors on other bacteria and mammalian cells) have been found to cooperate in activation of complement. Thus, pairs of antibodies, at concentrations of each which fail to activate complement, initiate the activation of this mediator system. The epitopes with which the antibodies in each of these pairs react must be within a spatial distance which permits bridging by a Clq molecule and subsequent activation of the complement sequence. The repeating epitope sequence of the type 2 fimbriae apparently differs considerably from that of the type 1 fimbriae since high concentrations of antibodies specific for the type 1 fimbriae activate complement whereas the majority of those specific for the type 2 fimbriae do not. The participation of both antibodies in the synergistic activation of complement was demonstrated by the finding that the combination of an $F(ab')_2$ fragment of one antibody with an intact antibody specific for a different epitope was ineffective. Also, binding of one antibody failed to significantly enhance the binding of the second antibody, a condition which could result in increased activation of the complement system.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00216-08 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Control of Connective Tissue Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Larry M. Wahl	Research Biologist	LMI, NIDR
Frederick Prosser	Postdoctoral Fellow	LMI, NIDR
Leroy L. Lampel		LMI, NIDR

COOPERATING UNITS (if any)

R. Wilder, NIAID
Dr. Reuben Siraganian, Chief, Clinical Immunology Section, LMI, NIDR

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human peripheral blood monocytes were isolated by counterflow centrifugal elutriation in a relatively unactivated state as accessed by the absence of collagenase production in control monocyte cultures. The addition of either concanavalin A (Con A) or lipopolysaccharide to the monocyte cultures resulted in the production of substantial levels of collagenase by these cells. Con A caused a more consistent activation with higher levels of collagenase. The amount of collagenase produced by the monocytes was 2 to 4 times that secreted by guinea pig peritoneal macrophages. Indomethacin inhibited Con A stimulated monocyte collagenase by at least 50% and this inhibition was reversed by 10^{-8} M PGE_2.

Examination of the production and regulatory influence on cell function of the known arachidonic acid (AA) metabolites has been made possible by the development of a high pressure liquid chemotography procedure which separates the individual prostaglandins, leukotrienes and monohydroxyeicosatetraenois acids (mono-HETE). Production of AA metabolites is being studied in lymphocytes, monocytes, basophils, platelets and polymorphonuclear leukocytes. The first results, obtained with the rat basophilic leukemia subline (RBL-1H3), have revealed that when these cells are stimulated with ionophore the ratio in AA metabolites released differs from that of IgE treated cells. Ionophore stimulated basophils release more total labeled material than antigen treated cells with the order of metabolites being 5 HETE>LTB4>PGD_2>PGE_2 whereas with antigen the ratio is PGD_2>PGE_2>5 HETE and LTB_4>HETE and LTE_4.

The study on the influence of the immune system on bone resorption has revealed that the spleen cells of osteopetrotic rats produce lower levels of IL-2 than those of normal littermates. This may, in part, account for the lower lymphocyte proliferative levels observed with lymphocytes of affected rats when compared to normal littermates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00254-07 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microbial Antigens Associated with Specific Adherence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John O. Cisar	Research Microbiologist	LMI, NIDR
Marcy Wax	Microbiologist	LMI, NIDR
Ann Sandberg	Chief, Humoral Immunity Section	LMI, NIDR

Stephan E. Mergenhagen, Chief, Laboratory of Microbiology and Immunology, NIDR

COOPERATING UNITS *(if any)*

W. B. Clark, University of Florida, F. C. McIntire, and A.E. Vatter, University of Colorado

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies are continuing on characterization of the lectin-carbohydrate interaction that mediates lactose sensitive coaggregation between oral actinomyces and streptococci. The lectin activity is a property of the type 2 fimbriae on strains of Actinomyces viscosus and A. naeslundii with lectin receptors on specific strains of Streptococcus sanguis and S. mitis. The introduction of A. viscosus chromosomal DNA into Escherichia coli using recombinant DNA technology and the isolation of clones expressing specific actinomyces antigens has provided an approach for the isolation of unassembled fimbrial subunits or precursor molecules. One clone has been found to produce a protein of approximately 60,000 daltons that reacts with monospecific and several monoclonal antibodies against type 2 fimbriae. A protein of similar size and antigenicity is present in A. viscosus cells and also has been detected as a subunit of partially dissociated type 2 fimbriae. An additional protein of approximately 35,000 daltons may also be involved in formation of the type 2 structure. In addition to studies of the type 2 fimbriae, immunochemical studies of S. sanguis 34 have associated a specific carbohydrate antigen with the receptor structure for lactose sensitive coaggregation. This antigen was detected in a mutanolysin digest of S. sanguis 34 cell walls but was not present on a spontaneous coaggregation defective mutant selected by its failure to react with certain D-galactose and N-acetyl-D-galactosamine binding plant lectins. The in vivo interaction of this receptor associated antigen with the type 2 fimbriae of actinomyces has been inferred from fluorescent double labeling of plaque samples using specific antibodies. Close and specific associations have been observed between actinomyces with type 2 fimbriae and streptococci labeled by monospecific antibody against the lectin receptor of S. sanguis 34. Such observations strongly support the concept that interbacterial adherence mediated by lectin-carbohydrate interactions is a central mechanism in the microbial colonization of teeth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00273-06

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Cell-Cell Interactions Between Oral Actinomyces and Other Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kolenbrander, Paul, E.	Research Microbiologist	LMI, NIDR
London, Jack	Research Microbiologist	LMI, NIDR
Ciardi, Joseph	Research Microbiologist	LMI, NIDR
Andersen, Roxanna	Microbiologist	LMI, NIDR
Tylenda, Carolyn	Research Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. A.L. Delisle University of Maryland, School of Dentistry Baltimore, MD
Dr. L.V. Holdeman VPI and SU Blacksburg, VA

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.80	2	.80

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Results from studies of coaggregation among oral bacteria during the past few years have built a firm foundation for future work. Bacteria isolated from subgingival and supragingival plaque have been examined and shown to exhibit a highly specific and ordered set of cell-to-cell interactions that forms a network of intergeneric cell surface recognitions. These cellular recognitions likely play a major role in the onset and development of bacterial plaque during the progressive changes in bacterial populations accompanying periodontal disease. We took advantage of this information to develop an in vitro model system of multiple cell-type coaggregations which simulates dental plaque. Several principles of coaggregation were predicted and demonstrable which give credence to the simulated plaque model. Results from related studies indicated that the oral bacteroides are particularly suited to be coaggregation bridge organisms because they are one of a few bacteria that recognize both oral streptococci or oral actinomyces and Actinomyces israelii. They also are able to be effective competitors against other bacteria for lactose-sensitive coaggregations with oral streptococci.

Coaggregation-defective mutants and bacteriophage-resistant mutants have been used as tools to unravel a sialic acid-inhibitable coaggregation that was complicated by being masked by other kinds of coaggregation mechanisms. The efforts of each of these investigative approaches and their results are focused on understanding the relationship of cell surface recognitions among oral bacteria and their role in microbial ecology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00290-5 LMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Production of Hybridomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.	Chief, Clinical Immunology	NIDR LMI
Hook, William A.	Research Microbiologist	NIDR LMI
Basciano, Luann K	Microbiologist	NIDR LMI
Berenstein, Elsa H.	Microbiologist	NIDR LMI
Fischler, Cynthia	Medical Technician Micro.	NIDR LMI
Garcia-Gil, Mercedes	Visiting Fellow	NIDR LMI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.65	2.15	2.50

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Hybridomas are being produced which secrete monoclonal antibodies of defined antigen specificity. Hybridomas have been produced against the Fc_ε receptor of mast cells and to human IgE. These monoclonal antibodies are being used for biochemical and biological studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00316-03 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Characterization and Biological Functions of Immunoregulatory Mediators in the Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Krakäuer, T.	Senior Staff Fellow	LMI, NIDR
Hallberg, P.	Bio Lab Technician	LMI, NIDR

COOPERATING UNITS (if any)

H. Jasin, University of Texas, Southwestern Medical School

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	.90	1.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human monocytes are activated by endotoxin and other inflammatory microbial products to produce hormone-like factors such as Interleukin 1 (IL-1). We have purified human IL-1 from peripheral blood monocytes to homogeneity using ultrafiltration, phenyl Sepharose chromatography, Ultrogel AcA54 chromatography and preparative isoelectricfocusing. Using purified samples of IL-1, we have determined that IL-1 is the mediator which activates chondrocytes to degrade cartilage matrix. We have also identified and cloned a human monocytic leukemia cell line (THP-1) which produces one of the two major components of IL-1. This cell line also produces a novel suppressor factor which inhibits the production and the action of Interleukin 2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE-00341-03 LMI

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)
Regulation of Sugar Transport and Metabolism in Lactic Acid Bacteria

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

John Thompson	Visiting Scientist	LMI, NIDR

COOPERATING UNITS (*if any*)
Bruce M. Chassy, LMI, NIDR
Jack London, LMI, NIDR
Sally Hausman, LMI, NIDR
William M. Egan, Div. of Biochemistry and Biophysics, Office of Biological Research Review, FDA
Dennis A. Torchia, Mineralized Tissue Res.Br. NIDR

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.4	1	.40

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type Do not exceed the space provided.*)

The hexose-6P: phosphohydrolase involved in the phosphoenolpyruvate -dependent futile cycle in *Streptococcus lactis* has been purified and characterized. Xylitol mediated inhibition of growth of *Lactobacillus casei* is due to the establishment of a PEP: pentitol futile cycle. Fourier transform, high resolution ^{31}P-NMR has been used to study sugar transport, and *in vivo* mechanisms for regulation of glycolysis, in physiologically intact cells of *S. lactis*. Lactose analogs have been prepared enzymatically, in which [^{14}C] 2-deoxy-D-glucose or [^{14}C] 2-fluoro-D-glucose replace glucose as the aglycon substituent of the disaccharide. The transport of the analogs by mannose: PTS and glucokinase defective mutants has been studied, and intracellular products have been identified. Evidence has been obtained for intracellular phosphorylation of glucose analogs by the mannose: PTS in streptococci.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00374-02 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Regulation of proliferation and differentiation of granulocyte/macrophage progenitor cells by Colony Stimulating Factors (CSF).

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dov H. Pluznik	Visiting Scientist	LMI, NIDR
Lynda Weedon	Biologist	LMI, NIDR

Stephan E. Mergenhagen, Chief, Laboratory of Microbiology & Immunology, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.50	1.50	1.

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Macrophages and granulocytes are involved in the inflammatory process. Proliferation and differentation of these cells from bone marrow hemopoietic stem cells (BMHSC) is regulated by the lymphokine colony-stimulating-factor (CSF). Because the percentage of stem cells in BM is very low, it is advantageous to use a dependent cell line to study how CSF regulates proliferation. Recently we described such a cell line, PT-18. In the present study we used this cell line to investigate the phase of the cell cycle in which CSF acts. Growth of cells was analyzed by staining them with propidium iodide and assaying DNA content by flow cytometry. Removal of CSF from the growth medium was followed by gradual accumulation of cells in the G_1 phase; 18 hr later, about 85% of the cells were in the G_1 phase. 10 hr after readdition of CSF, cells reentered the S phase. Furthermore, we found that CSF need not be present throughout the cell cycle; 6 hr of exposure of the cells cycle; 6 hr of exposure of the cells in G_1 to CSF were sufficient to drive the cells back into cycle. Cells arrested in G_1/S with aphidicolin and at G_2/M with colcemid, and then released from the arrest, proceeded in the cycle in the absence of CSF, but arrested again in an early stage of the G_1 phase. We conclude that CSF stimulates proliferation of cells by acting in the early G_1 phase. CSF may act on BMHSC in a similar way, driving the cells from G_0 into cycle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00381-01 LMI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Colonization of Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ciardi, Joseph E.	Research Biochemist	NIDR LMI
Kolenbrander, Paul E.	Research Microbiologist	NIDR LMI
Anderson, Roxanna N.	Microbiologist	NIDR LMI
McGray, Gina	Summer Fellowship Student	NIDR LMI
Lau, Agnes	Co-Step, Dental	NIDR LMI

COOPERATING UNITS *(if any)*

University of Oslo, Norway

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.6	1.00	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Cell-to-cell interactions between different genera of oral bacteria (coaggregation, interbacterial adherence) are considered of cardinal importance in the formation and persistence of tooth surface plaque. Studies on coaggregation between Actinomyces (coaggregation groups A-F) and oral streptococci (coaggregation groups 1-6) have identified 12 specific interaction patterns. The patterns are determined by the effect on coaggregation of heating or protease treatment of bacteria and after the addition of lactose to a coaggregating pair. The majority of the coaggregations identified are inhibited by lactose. The present study examined a lactose-inhibitable coaggregation that is highly specific in that it occurs between *Propionibacterium acnes* and only coaggregation group 1 streptococci; e.g. *Streptococcus sangius* DL1. Large, stable coaggregates formed and settled rapidly. Coaggregation was inhibited by lactose; of 10 galactosides tested, n-acetylglactosamine was the best inhibitor. Heating of the *S. sanguis* cells, but not the *P. acnes* cells, destroyed coaggregation ability. Coaggregation occurred in the presence of human saliva and was also inhibited by lactose. Saliva-induced aggregates of bacteria were easily differentiated from coaggregates by differences in size, stability and inhibition by lactose. In saliva, coaggregates formed at the expense of aggregates. Interbacterial adherence of *P. acnes* and *S. sanguis* also occurred on a saliva-coated surface. The attachment of the bacteria to the surface was not affected by lactose whereas the interbacterial adherence was inhibited. Similar results were obtained with saliva present in the liquid phase of the assay system. These findings further support the contention that very specific interactions involving lectins occur *in vivo* between different genera of oral bacteria and lead to the formation of unique bacterial compositions of supragingival and subgingival plaques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00382-01 LMI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Growth and Interaction of Oral Microorganism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Rohrish, Stanley A.	Reserach Microbiologist	NIDR LMI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The growth and physiologic interactions of two oral bacteria, Fusobacterium nucleatum and Streptococcus sanguis, are being investigated. The two organisms from a coaggregating pair with a characteristic morphology known as a "corn cob". A medium has been determined which should allow the growth of the Streptococcus on limiting glucose and the Fusobacterium on a limiting amino acid. A means for the continuous sampling of the culture during bacterial growth has been established. The fluorometric determination of DNA has been evaluated for a measure of growth. Initial experiments to determine the sequence of use of glucose and amino acids for energy by F. nucleatum have begun. Samples of the growth medium from F. nucleatum have been provided for assay of the characteristic effects of butyric acid in a tissue culture system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00383-01 LMI

PERIOD COVERED

October 1, 1983-September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biogenesis of Methane in Dental Plaque

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Christopher W. Kemp	Laboratory Technician	LMI, NIDR
Michael A. Curtis	Visiting Scientist	LMI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1	1.00	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is being conducted to demonstrate the presence of methanogenic bacteria in the dental plaque of monkeys (Macaca fascicularis and Macaca mulatta) and human periodontal patients. The work completed to date using enrichment culture techniques has demonstrated the presence and metabolic activities of these methanogenic bacteria and yielded human, methanogen isolates. The sork is being directed towards the taxonomic characterization of the pure cultures and the establishment of potential interactions between the oral methanogens and other strains of oral bacteria. The taxonomic experiments completed to date have established that the human isolates are Gram positive cocci in short chains. The isolates are metabolically and morphologically similar but immunologically distinct, (ABK1), from the genus Methanobrevibacter.

Preliminary results have been collected with respect to the participation of the human isolates in hydrogen and carbon transfer reactions. Binary mixtures of isolates ABK1 or ABK4 and Veillonella rodentium exhibited methanogenesis upon addition of lactic acid. Tertiary mixtures of ABK1, V. rodentium and oral saccharolytic bacteria resulted in methanogenesis when glucose was added to the mixture. Tertiary mixtures of ABK4, V. rodentium and saccharolytic strains revealed a utilization of ethanol as a methanogenic substrate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00384-01 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Effect of IgA1 protease on the biological activity of secretory IgA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael F. Cole	Visiting Scientist	LMI, NIDR
S. Dana Hsu	Laboratory Technician	LMI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Fourteen isolates of bacteria resembling Streptococcus sanguis have been obtained from the supragingival plaque of the monkey (Macaca fasicularis). The bacteria were identified by their enzyme activity, fermentation of sugars and production of glucan from sucrose. By use of fluorescent antibodies, specific for S. sanguis biotypes 1 and 2, all but one of the isolates appear to be biotype 1.

Secretory IgA has been purified from human colostrum by column chromatography and an IgA1 paraprotein from serum by HPLC. In addition, pools of colostrum and serum have been collected from M. fascicularis in order to obtain purified monkey 11S and 7S IgA.

The S. sanguis isolates appear capable of cleaving human IgA1 paraprotein as determined by cellulose acetate zone electrophoresis, immunoelectrophoresis and SDS polyacrylamide electrophoresis. The patterns of cleavage appear to differ from S. sanguis 10556.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00385-01 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lectin Dependent Adherence of Actinomyces to Human Epithelial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Brennan	Staff Fellow	LMI, NIDR
John Cisar	Microbiologist	LMI, NIDR
Ann L. Sandberg	Chief, Humoral Immunity Section,	LMI, NIDR

COOPERATING UNITS *(if any)*

Al Vatter

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies designed to identify the complementary structures on oral bacteria and mammalian cells which initiate attachment have demonstrated that adherence of Actinomyces naeslundii (WVU45) to several human epithelial cell lines is mediated by the lactose-sensitive fimbriae (type 2) of WVU45. This was determined by comparing the adherence of bacterial mutants which specifically lack the type 2 fimbriae and by using antibodies against the type 2 fimbriae which block attachment. The lectin-carbohydrate nature of the interaction was confirmed by specifically inhibiting attachment using β-galactosides and plant lectins which react with galactose or N-acetyl-galactosamine. These plant lectins which have specificities similar to that of the actinomyces fimbrial lectin are being used to identify the receptor on epithelial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00386-01 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Neuropeptides in Immune and Inflammatory Reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael F. Ruff	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neuropeptides, small signal peptides traditionally believed to function largely as transmitters of nerve impulses in the brain which mediate mood and emotion have now been shown to also regulate immune system function. Monocytes have receptors and will respond chemotactically to numerous neuropeptides. The doses for these reponses are very low, in the 10^{-14} M range, making this class of compounds among the most potent biological agents described. These results imply the existence of a neuroendocine link between the brain and the immune system whose purpose is to integrate behavioral and emotional responses with immune system function.

Additionally, we have shown that small cell lung cancer has surface antigens considered typical of macrophages. This disease may thus represent a dysfunction in the monocytic component of the immune system and may not be derived from lung cells as current dogma predicts. The neuropeptide bombesin, present in lung tumor cells, is chemotactic and growth promoting for these cells and may be involved in the pathogenesis of cancer as well as inflammatory diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00391-01 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Metabolism of the Oral Anaerobic Streptococci

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Curtis, Michael, A.	Laboratory Scientist (Vis)	LMI, Microbiology, NIDR
Kemp, Christopher, W.	Laboratory Technician	LMI, Microbiology, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology

INSTITUTE AND LOCATION

NIDR, NIH Bethesda MD

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is the examination of the amino acid and glucose metabolism of *Peptostreptococcus anaerobius*. This organism has been shown to have a unique nutritional relationship with the host salivary secretions: oral isolates have an absolute dependancy on the presence of proline in the medium for optimal growth; in its natural habitat the proline requirement is met by the proteins of the salivary secretions which contain as much as 30% of this cyclic amino acid. *P. anaerobius* ATCC 27337 has been shown to couple the reduction of proline to the oxidation of glucose and amino acids in Stickland-like reactions. The reductase activity towards proline was maximal in exponentially growing cells. Leucine reductase activity however was only found in stationary phase cells. No alterations to levels of these activities were detected in cells grown at different growth rates in a continuous culture apparatus. When grown in the absence of glucose alanine is the principle amino acid oxidised by this organism with the formation of acetic acid, ethanol and CO_2. Both non-glucose grown and glucose grown cells were able to metabolize this amino acid in washed cell suspensions. However alanine was shown to be formed as an end-product of the metabolism of glucose in the presence of ammonia by glucose grown cells. The catabolism and synthesis of alanine by *P. anarobius* is currently under further investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00392-01 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Monocyte Function in AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Phillip D. Smith, M.D. LMI, NIDR
Sharon M. Wahl LMI, NIDR
Kiyoshi Ohura, D.D.S.,Ph.D. LMI, NIDR
Terry Krakauer, Ph.D. LMI, NIDR

COOPERATING UNITS *(if any)*

Henry Masur Critical Case Medicine, Clinical Center
H. Clifford Lane LIR, NIAID
Anthony S. Fauci LIR, NIAID

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

National Institute of Dental Research

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1 .1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The monocyte-macrophage participates in many T- and B-lymphocyte functions and in the host response to many infectious organisms. To determine whether abnormal monocyte function occurs in the acquired immune deficiency syndrome (AIDS), a disease characterized by T- and B-cell defects and an unusually high incidence of certain protozoan, viral and fungal infections, we are investigating the expression of various functions by monocytes from patients with AIDS. Specifically, we are characterizing the ability of monocytes to exhibit chemotaxis, to express cytotoxic activity, to produce mediators such as prostaglandin E_2 and interleukin 1, and to generate microbicidal oxygen metabolites. Also, we are determining whether these monocyte activities can be augmented by immunomodulatory therapy. In addition, we are investigating the ability of cytomegalovirus (CMV) to infect monocytes and the effect of CMV infection on monocyte function. Finally, we will relate the findings from these studies to our clinical investigations into the microbiological manifestation of gastrointestinal disease in AIDS. These studies will provide insight into the participation of monocytes-macrophages in the immunosuppression in AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOL DE 00393-01 LMI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Macrophage Responses to Extracellular Organisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Phillip D. Smith, M.D.	LMI, NIDR
Sharon M. Wahl, Ph.D.	LMI, NIDR
Kiyoshi Ohura, D.D.S., Ph.D.	LMI, NIDR

COOPERATING UNITS *(if any)*

David B. Keister Laboratory Parasitic Dis., NIAID
Diane Taylor Georgetown University

LAB/BRANCH

Laboratory Immunology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project has been to characterize monocyte-macrophage responses to extracellular microorganisms as they relate to protective immunity. Using the extracellular protozoan Giardia lamblia as a prototype extracellular organism, we are investigating a) the mechanisms of monocyte chemoattration and cytotoxicity for G. lamblia; b) the ability of lymphocytes to regulate monocyte cytotoxicity of G. lamblia; and c) the effect of cytomegalovirus infection on monocyte responses to G. lamblia.

PHS 6040 (Rev. 1/84)

GPO 904-917

Laboratory of Oral Biology and Physiology

Summary Report

The Laboratory of Oral Biology and Physiology conducts basic research on the structure and function of secretory cells and tissues, and on specific enzyme-catalyzed posttranslational protein modifications. The Laboratory is divided into two research groups: the Experimental Morphology Section, which employs structural, biochemical and immunological approaches to investigate the secretory process in the salivary glands and pancreas; and the Enzyme Chemistry Section, which focuses on the mechanisms of action and physiological significance of the transglutaminases and their products, and the formation, fate and function of the amino acid hypusine and the protein in which it occurs. Although interactions between the two sections to date have been limited, largely because of their disparate interests and physical separation, it is anticipated that specific collaborative projects will be developed during the coming year. Each section, however, continues to make significant research advances in its own area of interest. Highlights of the progress during the past year are presented below.

Experimental Morphology Section

During the past year we have made significant new observations on the function of intralobular duct cells in the rat parotid gland. Employing a variety of protein tracers administered by retrograde ductal infusion, we have found that the intercalated and striated duct cells rapidly and avidly internalize certain tracers in large apical vacuoles. These vacuoles acquire acid hydrolase activity by fusion with lysosomes, and the enclosed tracers appear to undergo degradation. Other tracers are taken up in much smaller endocytic vesicles, and do not accumulate in large vacuoles. The mode of uptake apparently depends upon the chemical and physical properties of the tracer, and is not identical in the two types of ducts. The demonstration that the intralobular ducts are capable of endocytosing various exogenous proteins suggests that they may function to selectively reabsorb certain secretory proteins produced by the acinar cells. Other studies performed in LOBP indicate that reabsorption of "abnormal" secretory proteins may occur in experimental pathological conditions, such as diabetes and x-irradiation. Whether or not normal secretory proteins are also reabsorbed is as yet unknown. This newly discovered function expands the already known roles of the intralobular ducts in electrolyte secretion and reabsorption, and glycoprotein synthesis and secretion.

Endocytic phenomena have been further characterized in the exocrine pancreas using probes which bind to the cell membrane. The uptake of cationized ferritin essentially parallels that previously observed with soluble phase tracers, showing a different mode of uptake and sequestration, depending upon the cell surface involved. Significantly, as with all other tracers we have studied in the exocrine cells, no labeling of the Golgi saccules, GERL or immature secretory granules

occurred, thus raising questions about the routes, extent and significance of membrane reutilization in these cells. Initial results have also been obtained using monoclonal antibodies to acinar cell plasma membrane proteins. At the electron microscopic level, the antibodies were localized principally to the luminal membrane, using colloidal gold as an electron dense label. Studies on the localization of the antibodies following internalization should give significant new information on the processes of membrane retrieval, recycling and/or degradation.

Studies on the regulation of secretion in the rat parotid gland have demonstrated that forskolin, a diterpene which directly activates membrane adenylate cyclase, initiates a secretory response comparable to that elicited by the β-adrenergic agonist isoproterenol. Forskolin is highly effective in promoting amylase release and exocytosis of secretory granules in parotid acini incubated in vitro. Similar to the situation with isoproterenol, forskolin stimulation causes an intracellular redistribution of the regulatory subunits of cyclic AMP-dependent protein kinase: both R_I and R_{II} are associated with secretory granules in unstimulated cells, whereas R_I is significantly reduced in granule fractions prepared shortly after stimulation. These results confirm the role of the adenylate cyclase - cyclic AMP system as the mediator of protein secretion in the rat parotid gland. Additionally, the association of R_I and R_{II} with the secretory granules suggests that protein phosphorylation by the cyclic AMP-dependent protein kinase may be intimately involved in the process of exocytosis.

Although the role of the β-adrenergic system in the parotid gland is well established, studies of other salivary glands initiated during the past year indicate that its role in protein secretion is not universal. In vitro incubation of the lingual serous (von Ebner's) gland with cholinergic agonists results in the release of 15-20% of the lingual lipase and amylase activities over a 30 minute period, accompanied by extensive degranulation of the acinar cells. In contrast, with the β-adrenergic agonist isoproterenol, a maximum of 8% and typically less than 5% of the total lipase and amylase are released, and minimal exocytosis is observed in light microscopic sections. These results serve to emphasize the biochemical and functional diversity found in mammalian salivary glands, as well as the risks inherent in making generalizations from one tissue to another.

An important advance in the last few months is the establishment of appropriate culture conditions for the growth of isolated exocrine acinar cells. Although previous work had demonstrated that these cells could be maintained in vitro for several weeks, stimulation of proliferation of the differentiated secretory cells had not been achieved. By providing a substratum of basement membrane-like material, the exocrine cells attach to the dish, flatten, and within 48 hours, initiate DNA synthesis. The presence of secretory granules indicates that the cells retain their differentiated state. This newly developed system is expected to provide a wealth of opportunities to assess the regulation of cell growth, differentiation and function.

Enzyme Chemistry Section

The mechanism of action and biological functions of the class of enzymes known as transglutaminases represent a major part of the research interests of this Section. The use of a large number of synthetic peptides as substrates has revealed the requirements for specificity of the enzyme from various sources. These experiments also suggest that several specific enzyme inhibitors may be designed. A non-peptide inhibitor of transglutaminase, pentylamine phenylthiourea, has also been tested in vitro and in mice. These inhibitors have potential applications in transglutaminase-mediated pathogenic reactions, such as coronary thrombosis and disseminated intravascular coagulation.

Studies conducted during the past year suggest that the transglutaminases may have a much broader role in normal and pathological physiology than previously suspected. For example, the extracellular matrix of various tissues and organs has considerable associated transglutaminase activity, suggesting that transglutaminase-mediated protein cross-linking reactions may be important in matrix stabilization and tissue organization. The induction of histamine release from mast cells by IgE results in a significant covalent association of the histamine to protein that is most likely a result of intracellular transglutaminase activity. Increased levels of factor XIII(a_2) synthesized by the pregnant rabbit uterus, through the crosslinking of cell surface proteins, may be involved in the suppression of spermatozoal antigenicity. Coupled with the extensive knowledge of the mechanisms of enzyme action gained through many years of basic biochemical investigations, these current studies are beginning to provide an understanding of the important biological functions of the transglutaminases.

Investigations during the last few years have demonstrated the formation of the unusual amino acid, hypusine, as a posttranslational modification in a single protein in all mammalian cells examined. Hypusine is formed from protein-bound lysine and the butylamine portion of the polyamine, spermidine. The protein in which it occurs is eukaryotic translation initiation factor-4D. Although the in vivo function of eIF-4D has not been established, this specific modification which occurs in growing cells is likely to be highly significant in relation to the biological activity of the protein. Research this last year has concentrated on completing the biochemical details of the formation of hypusine, and on the production of antibodies to eIF-4D. The development of inhibitors of hypusine biosynthesis, and employment of antibodies to eIF-4D in protein synthesis experiments, will be the initial steps in understanding the biological role and mechanism of action of this protein.

PUBLICATIONS

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Bodner, L., Kuyatt, B.L., Hand, A.R. and Baum, B.J.: Rat parotid cell function in vitro following x-irradiation in vivo. Radiat. Res. 97:386-395, 1984.

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Doine, A.I., Oliver, C. and Hand, A.R.: The Golgi apparatus and GERL during postnatal differentiation of rat parotid acinar cells: An electron microscopic cytochemical study. J. Histochem. Cytochem. 32:477-485, 1984.

Field, R.B. and Scow, R.O.: Purification and characterization of rat lingual lipase. J. Biol. Chem. 258:14563-14569, 1983.

Hand, A.R. and Oliver, C.: Effects of secretory stimulation on the Golgi apparatus and GERL of rat parotid acinar cells. J. Histochem. Cytochem. 32:403-412, 1984.

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Mednieks, M.I. and Hand, A.R.: Microheterogeneity of rat parotid gland proteins after chronic treatment with isoproterenol. J. Dent. Res. 63:87-93, 1984.

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Park, M.H., Chung, S.I., Cooper, H.L. and Folk, J.E.: The mammalian hypusine-containing protein eIF-4D. Structural homology of this protein from several species. J. Biol. Chem. 259:4563-4565, 1984.

Qwarnstrom, E., Bodner, L., Baum, B., Hand, A. and Omnell, K-A.: Saliva secretion from the rat submandibular gland after retrograde infusion of radiographic contrast media. J. Dent. Res. 63:614-617, 1984.

Qwarnstrom, E.E. and Hand, A.R.: A morphologic study of the recovery of the rat submandibular gland after retrograde infusion. II. Lipid-soluble radiographic contrast medium. J. Oral Pathol. 12:430-445, 1983.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z 01 DE 00001-32 LOBP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Specificities, Physiological Functions and Biological Roles of Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Folk, J.E.	Chief, Enzyme Chemistry Section	LOBP NIDR
OTHERS:	Thacher, S.	Staff Fellow	LOBP NIDR
	Fesus, L.	Guest Researcher	LOBP NIDR
	Beninati, S.	Guest Researcher	LOBP NIDR
	Piacentini, M.	Guest Researcher	LOBP NIDR

COOPERATING UNITS *(if any)*

Dr. Jeffrey J. Gorman, University of Melbourne, Parksville, Victoria, Australia; Dr. Mary Lynn Fink, Baylor University, Waco, Texas

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.0	2.4	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the specificity and catalytic mechanism of transglutaminases are underway. The cellular and extracellular functions of the products of transglutaminase action are under investigation. Knowledge has been obtained as to the catabolic fate of these transglutaminase products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z 01 DE 00028-17 LOBP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ultrastructure and Cytochemistry of Secretory Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Hand, A.R.	Chief, LOBP	LOBP NIDR
OTHERS:	Oliver, C.	Research Biologist	LOBP NIDR
	Mednieks, M.I.	Senior Staff Fellow	LOBP NIDR
	Coleman, R.	Visiting Scientist	LOBP NIDR
	Mazariegos, M.R.	Visiting Fellow	LOBP NIDR
	Hani, S.	Guest Researcher	LOBP NIDR
	Baum, B.J.	Chief, CIPCB	CIPC NIDR
	Kousvelari, E.	Expert	CIPC NIDR
	Lustmann, J.	Guest Researcher	LOBP NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.62	2.97	2.65

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basic mechanisms of the secretory process are studied in cells of the rat pancreas, salivary and lacrimal glands. Techniques utilized include light and electron microscopy, cytochemistry, radioautography, and biochemistry. Major areas of investigation are: (1) the structure and function of the Golgi apparatus and GERL; (2) experimental pathology and lysosome function in salivary glands; (3) structure and permeability properties of junctional complexes in the rat parotid gland; (4) the role of salivary duct cells in protein reabsorption; (5) structure and cytochemistry of the salivary glands of the rat soft palate; and (6) parotid β-adrenergic receptor localization using labeled antagonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z 01 DE 00049-13 LOBP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Function of Transglutaminases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Chung, S.I.	Research Chemist	LOBP NIDR
OTHERS:	Cocuzzi, E.T.	Visiting Fellow	LOBP NIDR
	Fesus, L.	Guest Researcher	LOBP NIDR
	Kim, H.	Guest Researcher	LOBP NIDR
	Lee, K.R.	Guest Researcher	LOBP NIDR

COOPERATING UNITS (if any)

Dr. F. Carmassi, University of Pisa; Dr. D. Galanakis, SUNY, Dr. S.Y. Lee, Catholic Medical College, Seoul, Korea; Dr. A. Mukerjee, NEI.

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.35	3.2	0.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The physiological function and the mode of regulation of transglutaminases are being studied as to their role in the formation of temporary tissue matrix (fibrin-connective tissue matrix) during tissue or bone fracture repair and in the modulation of specific cellular processes.

The physiological significance and biochemical mechanism of factor XIIIa (plasma transglutaminase) and tissue transglutaminase catalyzed cross-linking of plasmin inhibitor (α_2PI) to fibrin and other matrix proteins are under investigation both *in vivo* and *in vitro*. The cross-linking of α_2PI to fibrin plays a major role in stabilization of a temporary matrix which is vital for the initial phase of cell miigration and proliferation at wound sites. A number of factors in the plasma and tissue fluid can effect the fibrin stabilization, i.e., glutathione, superoxides, and metal ions. We now find albumin plays an important role in the modulation of both fibrin gel-formation and stabilization.

Factor XIII (a_2) has been isolated from human uterus and placenta but its origin is not known. By immuno-histological methods, the increased level of Factor XIII (a_2) antigen was observed in the myometrium and endometrium of the pregnant rabbit uterus and a concomitant increase of thrombin-dependent transglutaminase activity was also detected in the same tissues.

In cells activated by mitogens and in transformed cells, various molecular forms of transglutaminases have been identified. In the lung and muscle, most of enzyme activities are associated with non-extractable insoluble matrix proteins whereas in the liver and kidney, the majority of enzyme activity is found in the cytosol fractions. The intrinsic substrates for the extracellular matrix-bound enzymes are also localized in the same proteins.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z 01 DE 00199-08 LOBP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

In Vitro Studies of Secretory Cell Structure and Function

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Oliver, C.	Research Biologist	LOBP NIDR
OTHERS:	Hand, A.R.	Chief, LOBP	LOBP NIDR
	Livne, E.	Visiting Fellow	LOBP NIDR
	Ogrowsky, D.	Visiting Fellow	LOBP NIDR
	Dromy, R.	Guest Researcher	LOBP NIDR
	Kleinman, H.	Research Chemist	LDBA NIDR
	Siraganian, R.	Chief, CI, LMI	LMI NIDR

COOPERATING UNITS (*if any*)

Dr. A. Robbins, GB, NIADDK

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
5.13	2.53	2.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Secretory and endocytic processes in several cell types are currently under investigation. Cell dissociation and short term culture (up to 1 month) methods have been established for rat exorbital lacrimal, parotid and pancreatic acinar cells. These cultures are being used to study various aspects of the secretory process. Emphasis is placed on morphological, cytochemical and biochemical characterization of the cultured cells. Uptake and fate of both soluble phase and membrane bound markers by exocrine acinar cells are being examined in vivo and in vitro. The lysosomal system and its role in endocytic processes is also under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z 01 DE 00217-06 LOBP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Salivary Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Wolf, R.O.	Dental Director	LOBP NIDR
OTHERS:	Kingman, A.	Statistician (Health)	CP P NIDR

COOPERATING UNITS *(if any)*

Hubbard, V.S.	Physician	PM NIADDK
Merril, C.	Physician	LGCB NIMH

LAB/BRANCH
Laboratory of Oral Biology and Physiology

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with mechanisms of production and control of extrinsic (i.e., saliva) and intrinsic (e.g., serum salivary isoamylase) salivary gland products. Human and animal saliva chemical constituents and mechanisms are evaluated in relation to health, disease and physiological state. Parotid salivary flow rate, protein content and enzymes are evaluated in normal subjects and in selected disorders. The intrinsic secretion of salivary isoamylase in serum of patients with cystic fibrosis of the pancreas has been studied. Diagnostic applications are being sought.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00285-04 LOBP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Protein Secretion in the Rat Parotid

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mednieks, M.I.	Senior Staff Fellow	LOBP NIDR
OTHERS:	Cheng, L.F.	Visiting Fellow	LOBP NIDR
	Hand, A.R.	Chief, LOBP	LOBP NIDR
	Siraganian, R.	Chief, CI	LMI, NIDR

COOPERATING UNITS *(if any)*

Prof. R.A. Jungmann, Northwestern University; Prof. F.J. Dowd, Creighton University; Dr. E. Watson, University of Washington.

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms of action of protein synthesis and secretion were studied in acinar cells of the rat parotid. Monoclonal antibodies to α-amylase purified from rat parotid secretory granules were purified and characterized using chromatographic and solid phase immunosorbent (ELISA) techniques. Preparation of mRNA from the salivary gland was carried out and measurement of total and specific (immunoreactive with anti α-amylase reagent) translation products served as an index of protein synthetic responses to in vivo β-adrenergic stimulation of the animal with isoproterenol. The pharmacologic agent forskolin was useful for in vitro stimulation of parotid acinar tissues. The effects of the secretagogue on cellular signal processing were determined:

a) morphologically (LM, EM)
b) biochemically (cAMP-dependent protein phosphorylative reactions and specific enzyme distributions).

Exocytosis can be regulated by agents which either undergo receptor interactions or involve cell membrane adenylate cyclase directly. Mechanisms of action of these stimuli appear to be cyclic AMP-dependent reactions involving gene regulation by cyclic AMP-mediated phosphorylative modification of variously compartmentalized cellular proteins. Physiologically these reactions are cellular manifestations of stress responses in general, but specifically involve oral soft tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z 01 DE 00311-04 LOBP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

The Unusual Amino Acid, Hypusine: Mechanism of Formation and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Folk, J.E.	Chief, Enzyme Chemistry Section	LOBP NIDR
OTHERS:	Park, M.H.	Staff Fellow	LOBP NIDR
	Abbruzzese, A.	Visiting Fellow	LOBP NIDR

COOPERATING UNITS *(if any)*

Dr. H.L. Cooper, NCI, LPP; Drs. D.J. Liberato and A.L. Yergey, NICHHD, NPMB

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.8	2.45	0.35

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The amino acid hypusine has been identified as a component of a single protein in all mammalian cells examined. This protein is eukaryotic translation initiation factor 4D (eIF-4D). Hypusine is formed posttranslationally from lysine and the butylamine portion of the polyamine, spermidine, followed by hydroxylation. These data demonstrate a novel polyamine metabolic pathway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z 01 DE 00376-01 LOBP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Secretion, Purification and Charcterization of Enzymes from Lingual Serous Glands.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Field, R.B.	Staff Fellow	LOBP NIDR
OTHERS:	Hand, A.R.	Chief, LOBP	LOBP NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20205

TOTAL MAN-YEARS.	PROFESSIONAL	OTHER
1.9	1.15	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to investigate the secretory process in von Ebner's gland of the rat tongue, we are studying the effects of cholinergic and α- and β- adrenergic agonists and antagonists on the secretion of lingual lipase and amylase. The amount of secretion is determined by in vitro incubation of the dissected glands with the agonists. The tissue and medium are assayed for lipase and amylase activity and portions of the tissue are taken for light and electron microscopy.

Lingual lipase and amylase were purified from von Ebner's gland. The purification is being scaled up to produce enough purified lingual lipase to prepare antibodies and to further characterize the enzyme by isoelectric-focusing and amino acid and carbohydrate analyses.

PHS 6040 (Rev. 1/84)

GPO 904-917

Laboratory of Developmental Biology and Anomalies Summary Report

A site visit to the Laboratory of Developmental Biology and Anomalies by the Board of Scientific Counselors and ad hoc representatives was held on December 7-9, 1983. In general, the Site Visitors were pleased with the progress and quality of the work in LDBA. They noted that the Sections currently in LDBA, the Craniofacial Development Section and the Connective Tissue Section, due to changes in staff and program no longer represent the major research foci in the Laboratory. They recommended that these organizational units be abolished and that new units be created with time as justified. These sectional designations have now been disbanded and a proposal to create a Cell Biology Section in LDBA has been made. Some program changes are being made with the departure or impending departure of staff (Varner to Baylor College in Texas; Terranova to Revlon Corp, Rockville; Grotendorst to Univ of S. Carolina; Chandrasekhar to Eli Lilly Co, Indianapolis; Ledbetter to Upjohn, Kalamazoo, Young to MTRB, NIDR). Various new people will be joining the Laboratory including Bolander (an orthopedic surgeon), Martinet (a dermatologist), Killen (an internist), Kitten (a cell biologist), Noonan (a cell biologist), and Horton (a teratologist). These staff changes will lead to new directions in these programs. There will be a much greater emphasis on molecular biology approaches to studying connective tissue structure, function and pathology. The success of our molecular biology group was noted by the Site Visitors and increased focus was recommended as a desirable course.

Two members of LDBA have received recognition for their achievement during the past year. Dr. Hynda Kleinman received the NIH Director's Award "for characterizing protein factors in connective tissues which specify the interaction of cells with matrix and regulate their form, function and repair". Dr. George R. Martin received the Distinguished Service Award of DHHS "for outstanding basic research that has significantly advanced our understanding of biological processes critical to the development, maintenance and repair of normal tissues".

Research Accomplishments

Studies on the Structure and Regulation of Connective Tissue Genes

Cartilage collagen - Good progress has been made on cloning type II collagen. Previously we had obtained clones for the 3' portion of the chain. Now we have isolated a cDNA clone to the 5' region of the mRNA and the amino acid sequence of this portion of the chain has been deduced for the first time. Further, we have isolated genomic clones that code for this portion of the rat gene including the promoter. Some 1000 bases of the promoter and the first exon have been sequenced. Unique features of the promoter include the lack of a CAT box and the presence of a repeating structure that has been identified previously as a control element in a viral gene. Further studies are planned with the promoter of this gene such as testing for tissue-specific DNA binding proteins. Using these rat probes, we have screened for and obtained DNA clones containing analogous portions of the human gene for type II collagen. The structure of these clones is being studied. In addition, these clones are being used in a collaborative study with Johns Hopkins Hospital scientists to screen the DNA from patients with inherited diseases of cartilage for alterations in gene structure. We have

also examined the regulation of expression of collagen genes. The methylation pattern for the α 1(II) chain gene in cells that express or do not express this protein has been studied. We find that this cartilage gene is more highly methylated in fibroblasts and in liver but is hypomethylated in chondrocytes. Dedifferentiated chondrocytes while not synthesizing the protein, also maintain the gene in a hypomethylated state.

Type I and type II collagen genes have been used to examine the structure of collagen genes in rat liver from young and aged animals. These studies indicate that there are no general rearrangements in the primary structure of the gene. However, we have noted that these genes become hypomethylated in the aged tissue. Based on the current concept that hypomethylation is correlated with gene expression, it is possible that this change represents a general and significant alteration in the genome with aging.

Basement Membrane Genes

Clones coding for some 50,000 bases of the gene for the pro α1(IV) chain have been isolated in this Laboratory. Examination of heteroduplexes between these clones and the mRNA for the chain show that the majority of the coding portions of the gene occur as many small DNA segments. Similar observation have been made for interstitial collagen genes where the helical portion of the gene is divided into 54 bp segments. It has been proposed that the collagen gene arose by the duplication of a primordial 54 bp gene. However, we have sequenced two such segments in the pro α1(IV) chain and these contain some 170 bases. These findings suggest independent evolution of these genes.

Extracellular Matrices and Cell Biology

Basement membrane structure and assembly - Heparan sulfate is the major glycosaminoglycan in basement membrane where it regulates the filtration of proteins. We have isolated the major heparan sulfate proteoglycans from basement membrane and found that they occur in two forms: a large, tightly associated proteoglycan and a smaller, readily extracted form. The nature of these proteoglycans has been investigated by electron microscopy, by immunological methods and by biosynthetic experiments. The larger proteoglycan is the precursor of the smaller one and it represents the functional form in the basement membrane.

The assembly of basement membrane occurs by specific interactions among its components, particularly type IV collagen, laminin, the large heparan sulfate proteoglycan as well as nidogen and entactin. Our recent studies indicate that these components form a supramolecular complex of defined composition which is the intermediate in basement membrane formation. This complex has been called the basement membrane matrisome. These reconstituted basement membranes have been used as substrates to culture various cells.. Epithelial cells show enhanced survival, proliferation and growth in culture on this matrix.

Metastatic Tumor Cells

We have found that the interaction of metastatic tumor cells with basement membrane differs from that of normal cells or benign tumor cells.

The metastatic cells show a higher affinity for basement membranes due to the presence of an increased number of laminin receptors on their surface.

We have developed an artificial basement membrane which acts as a barrier to migration when cells are placed on it to study the invasive properties of these cells. Metastatic cells pass through the barrier at a rate proportional to the metastatic activity that they demonstrate in vivo whereas nonmetastatic cells do not penetrate this barrier. Using this system, we have also tested for tissue-specific factors that might attract metastasizing cells and thus explain why certain tumors are organ-specific in their metastases. In these studies, we investigated tumor cell strains that metastasize to either brain, lung or liver. We found that the tissue to which each cell metastasizes contains a chemoattractant specific for that cell strain but not for the others. This artifical basement membrane barrier should prove useful for characterizing the factors responsible for organ-specific metastases as well as for studying the nature of the tumor cells. It also may be useful as a system for screening drugs specific for metastatic cells.

Wound Healing

Factors that may promote the rate of wound healing have been investigated. These studies show that supplementing wounds with the platelet-derived growth factor (PDGF) leads to a near doubling of the rate of collagen deposition. PDGF attracts fibroblastic cells to the wound and then induces their proliferation. Wound healing has also been investigated in two pathological states, diabetic rats and rats treated with cytostatic agents. Wound healing is reduced in these situations but restored to normal with PDGF supplements suggesting that the amount of PDGF may be limiting the rate of wound healing. These studies indicate wound healing can be increased by external supplements. The possibility of stimulating the healing of mineralized tissues is under investigation.

Developmental Biology

Animal studies with jervine and cyclopamine, alkaloids derived from certain poisonous plants, have shown that they are potent teratogenic substances preventing normal cartilage and bone development. When tested on differentiating limb bud cells, we found that the stem cells are killed by these alkaloids, whereas the differentiated cells are resistant. Similar results were obtained using muscle fibroblasts which are induced to differentiate into cartilage by bone morphogenic protein. These results indicate that jervine and cyclopamine show marked selectivity for undifferentiated cells. Their mechanism of action is under study.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00006-24 DB

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemotaxis in Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Schiffmann, Elliott	Research Chemist	NIDR DB

COOPERATING UNITS *(if any)*
NCI, NIH; NINCDS, NIH

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION
Connective Tissue Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A principal feature of metastatic tumors is their invasiveness. Cells detaching from primary tumors must cross membrane barriers to reach secondary target sites. Our studies are focussed upon an aspect of the invasive process; namely, the chemotactic responsivenss of tumor cells to certain chemical stimuli. Using a human melanoma cell line we have found that these cells migrate in response to a material in their conditioned media which has an M_r of about 70KD and does not appear to be identical to other known attractants. The material may have plasminogen activator activity. These melanoma cells also respond to the peptides F-Met-Leu-Phe and bombesin. Additionally, we have found that a highly metastatic murine melanoma cell line selected from a subline of cells is markedly more chemotactically responsive than a poorly metastatic line from the same subline.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00009-23 DB

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Chemistry and Biosynthesis of Connective Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Martin, George R.	Ch, Lab. Dev. Bio. & Anomalies	NIDR DB
Kleinman, Hynda K.	Research Chemist	NIDR DB
Wagner, Clayton	Biologist	NIDR DB

COOPERATING UNITS *(if any)* Max-Planck-Institute for Biochemie, Germany; McGill University, Canada; University of Texas, San Antonio, Texas

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION
Connective Tissue Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.05	3.25	1.80

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the formation, function and the destruction of connective tissue components in normal and diseased states. Particular attention is directed toward various matrix molecules including collagens, glycoproteins, and proteoglycans. Current aspects of this project include (1) characterization of the interactions between matrix molecules, (2) their effect on cell growth and differentiation and (3) DNA changes in various pathological conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00024-18 DB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental processes in genetically controlled malformations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, Kenneth S.	Medical Director	NIDR DB
Campbell, Marlissa	Postodoctoral Fellow	NIDR DB
Harne, Leslie C.	Bio Lab Technician (Animal)	NIDR DB

COOPERATING UNITS *(if* Howard University; University of Maryland; University of Washington, Seattle; NCI, NIH; NEI, NIH; NIAMDD, NIH; NIEHS, NIH; and USDA Poison Plant Laboratory

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER.
5.90	1.85	4.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are using mice with genetic defects in craniofacial development or differences in susceptibility to teratogens as models to study the mechanisms involved in craniofacial malformation. Mutations that cause cleft lip, cleft palate, malocclusion and exencephaly have been found to have chondrocytes that produce altered cartilage matrix. Several different genetic defects in matrix biochemistry result in similar malformation syndromes. Other mutant genes reduce or render defective essential cellular processes such as keratinization or other types of cell differentiation with resultant malformation. Other genes that modify the action of teratogenic drugs are used to explore the mechanisms of teratogenesis and to develop sensitive test systems for recognition of teratogens. These mouse genetic test systems are produced using uniform, highly inbred, genetically defined strains and timed mating resulting in control of stages of development for tests of target tissues at critical periods of susceptibility in vivo or in vitro. Organs or organ primordia such as limb buds or cells from embryos of appropriate genetic type and developmental stage are labeled in vivo or in vitro with isotopic precursors for specific structural molecules in order to determine the mechanisms of gene action in development and the mechanisms of teratogenesis.

These methods have proven successful in identification molecular defects in cartilage matrix and other specific molecular errors that result in malformation. We are continuing to use this approach to determine the molecular vocabulary of development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00025-18 DB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of connective tissue gene expression during development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Yamada, Yoshihiko	Visiting Scientist	NIDR DB
Doege, Kurt	Biologist	NIDR DB
Fernandez, Pilar	Visiting Fellow	NIDR DB
Nunez, Anne Marie	Visiting Fellow	NIDR DB
Sakurai, Yoshiki	Visiting Fellow	NIDR DB
Kohno, Kimitoshi	Visiting Fellow	NIDR DB
Gershon, David	Visiting Scientist	NIDR DB
Martin, George R.	Ch, Lab. Develop. Biol. & Anomalies	NIDR DB

COOPERATING UNITS (if any)

Max-Planck Institute for Biochemie; Genex Corporation; John Hopkins University; NICHD, NIH; NIADDK, NIH; NEI, NIH

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
10.27	6.90	2.37

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. We have constructed a cDNA clone complementary to the 5' terminal portion of the rat type II collagen mRNA. This clone contains a 521 bp cDNA insert including 153 bp of the 5' untranslated region plus 368 bp coding for the signal peptide, the amino propeptide and a part of the telopeptide. 2. We have isolated several genomic clones which contain the 5' terminal portion of the rat type II procollagen gene. We determined the nucleotide sequence of the promoter and the first exon of the gene. 3. We have constructed a cDNA clone coding for the mouse pro α1 type IV collagen chain. DNA sequence analysis showed that the globular domain of the pro α1(IV) chain is composed of 273 amino acids. 4. We have isolated several overlapping genomic clones which span about 40 kb and contain much of the mouse pro α1(IV) collagen gene chain. The coding region of this gene is subdivided into many small exons, roughly similar in size and each estimated to be about 170 bp. Sequencing of one of the exons shows it to be 178 bp in length and to code for a helical segment of the protein. The difference in exon size for helical domains between the pro α1(IV) and the chains for types I, II and III collagen suggest that this basement membrane gene evolved independently of the interstitial collagen genes. 5. We have isolated almost the complete human type II collagen gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00149-10 DB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alterations in Proteoglycans During Abnormal Development and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hassell, John R.	Research Biologist	NIDR DB
Ledbetter, Steven	Guest Researcher	NIDR DB
Kleinman, Hynda K.	Research Chemist	NIDR DB
Martin, George R.	Ch, Lab. Develop. Biol. & Anomalies	NIDR DB
Leyshon, Webster	Biologist	NIDR DB
Mosley, General	Biol. Lab Tech.	NIDR DB
Horigan, Elizabeth	Biologist	NIDR DB

COOPERATING UNITS *(if any)*

NCI, NIH; St. Lukes Medical Center, Chicago, Ill.; Montreal Childrens Hospital, Montreal, Canada; Scripps Clinic and Research Foundation, La Jolla, California

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Craniofacial Development Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.40	2.80	3.60

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to understand the role that proteoglycans play in the structure and function of tissues and during developmental events. We are characterizing tissue specific proteoglycans and determining the metabolic steps involved in their synthesis. Particular attention has been directed toward studying the heparan sulfate proteoglycan of basement membranes. We have identified the precursor protein which serves as a specific acceptor for the addition of heparan sulfate chains and established that several forms of the proteoglycan exist due to processing. Only one form, the largest, interacts strongly with other matrix components and the others may arise by degradation.

We are also studying alterations in proteoglycan synthesis in human diseases such as diabetes and macular corneal dystrophy. In diabetes, there is a reduction in the synthesis of the basement membrane proteoglycan which produces basement membrane thickening due to compensatory hypertrophy. In macular corneal dystrophy, there is a failure to synthesize a mature corneal proteoglycan which results in corneal clouding and eventually blindness. We are also using mouse models to determine the mechanism by which teratogens disrupt the synthesis of the cartilage proteoglycan during chondrogenesis and of the basement membrane proteoglycan during myogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00230-08 DB

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of extracellular matrix proteins in tissue architecture and cell function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kleinman, Hynda K.	Research Chemist	NIDR DB
Chandrasekhar, S.	Guest Researcher	NIDR DB
Martin, George R.	Ch, Lab. Dev. Biol. & Anomalies	NIDR DB
Hassell, John	Research Biologist	NIDR DB
Laurie, Gordon	Visiting Fellow	NIDR DB
Cannon, Frances	Biologist	NIDR DB
Aumailley, Monique	Visiting Fellow	NIDR DB

COOPERATING UNITS *(if any)* NIAID, NIH; NCI, NIH; NINCDS, NIH; University of Minnesota; Veterans Administration; Georgetown University; McGill University, Montreal, Canada; Rutgers University; Albert Einstein College of Medicine

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION
Connective Tissue Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
6.63	3.50	3.13

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the structure and function of extracellular matrix components. Our data define how the components of skin, cartilage and basement membrane interact with each other to assemble into supramolecular complexes. The basement membrane collagens, proteoglycans, cell attachment proteins and other glycoproteins of the respective tissues bind to each other in a highly specific manner and in precisely defined stoichiometry to form ordered complexes. These interactions define the structure and function of the matrix in tissue morphogenesis, in wound repair and in certain diseases.

Various biological activities of epithelial cells are controlled by basement membrane components. The adhesion and growth of neonatal Schwann cells is specifically increased several-fold by laminin and the cells appear more differentiated and elongated. B16 Melanoma cells produce more pigment in vitro when cultured on basement membrane substrates. These and other studies on cultured cells indicate that the matrix components may regulate tissue formation and repair by determining which cells attach and proliferate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00253-06 DB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of cartilage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Varner, Hugh H.	Staff Fellow	NIDR DB
Jerdan, Janice	Postdoctoral Fellow	NIDR DB
Horn, Valerie	Biologist	NIDR DB
Lee, Willard	Chemist	NIDR DB
Martin, George R.	Ch, Lab. Develop. Biol & Anomalies	NIDR DB
Prabhakar, Bellur		NIDR OM

COOPERATING UNITS *(if any)*

University of Iowa, University of Minnesota, Walter Reed Army Institute of Research, Worcester Foundation for Experimental Biology

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.20	2.35	1.85

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously we have isolated a glycoprotein, termed chondronectin, from chicken serum which was utilized by chondrocytes to bind to collagen. Immunological studies showed that the protein was a cartilage specific protein occuring in the matrix around the chondrocytes. Further studies showed that chondronectin binds both to collagen and to the glycosaminoglycan chains of the chondroitin sulfate proteoglycan of cartilage. The affinity of chondronectin for various glycosaminoglycans has been studied and the binding of chondronectin to dextran has been used for the affinity purification of the protein. Using this method, we ave recently isolated chondronectin from human serum. Human chondronectin is very similar in size, chain structure and biological activities. Antibody has been prepared to human chondronectin and used to develop an assay for chondronectin in human serum. It is possible that serum chondronectin levels will parallel the metabolic activity of cartilage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00275-06 DB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological testing of fluoride

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Martin, George R.	Ch. Lab. Deve. Bio. & Anomalies	NIDR DB
Brown, Kenneth S.	Medical Director	NIDR DB

COOPERATING UNITS *(if any)*

University of Minnesota; NCI, NIH

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.25	.20	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the action of fluoride in various biological tests used to detect clastogenic or mutagenic substances. To date, fluoride has been examined in tests used to detect mutagens and found to be non mutagenic. No effects on chromosome structure were noted in animals given widely different levels of fluoride. DNA repair after X-ray was unchanged by fluoride. No genetic effects of fluoride were noted in a recessive lethal test of fluoride on Drosophila. The data indicate that fluoride has no mutagenic activity. Reports of fluoride's action on the metabolism and growth of cells are being monitored and where appropriate, related studies are undertaken.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00330-03 DB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of attachment proteins in tumor cell metastasis and periodontal reattachment

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Terranova, Victor P.	Research & Clinical Associate	NIDR DB
Hujanen, Errki	Visiting Fellow	NIDR DB
Schiffmann, Elliott	Research Chemist	NIDR DB
Kleinman, Hynda	Research Chemist	NIDR DB
Martin, George R.	Ch, Lab. Develop. Biol. & Anomalies	NIDR DB

COOPERATING UNITS *(if any)* NCI, NIH; NIMH, NIH; NIAID, NIH; Georgetown University; Eastman Dental Clinic; University of Rochester, M.D. Anderson Hospital and Tumor Institute; National Naval Medical Center; Frederick Cancer Research Facility; University of Buffalo; Helitrex, Inc.; Meloy Laboratories; Revlon Biotech. Research Center; University of Texas; Johns Hopkins University

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.46	2.55	1.91

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the interaction of normal and metastatic tumor cells with components of the extracellular matrix. Specific emphasis has been placed on understanding metastatic tumor cell usage of laminin, various laminin fragments and the laminin receptor in cell adhesion to basement membrane. Additional studies have included the examination of directed migration of metastatic tumor cells to target organ specific factors. These studies have been termed "chemoinvasion" (chemo from chemotaxis and invasion from the cells ability to invade an artifical basement membrane barrier). Using the artificial basement membrane barrier various tumor cells have been selected for their ability to invade and migrate. These cells show increased metastatic propensity. Further studies have utilized laminin and fibronectin to modulate of the invasive potential of tumor cells. Laminin increases, while fibronectin decreases invasiveness.

A second direction of this project involves laminin and fibronectin in conjunction with tooth root preparation as a mean to stimulate periodontal reattachment. Various root preparations are being considered.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00331-03 DB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Connective Tissue Cell Chemoattractants in Wound Healing and Fibrotic Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Grotendorst, Gary R.	Staff Fellow	NIDR DB
Harvey, Anity K.	Guest Researcher	NIDR DB
Martinet, Nadine	Guest Researcher	NIDR DB

COOPERATING UNITS *(if any)*

NCI, NIH; NEI, NIH; USUHS

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Connective Tissue Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.83	2.15	1.68

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are continuing our studies on factors which control the migration and proliferation of connective tissue cells. We have shown that the platelet-derived growth factor (PDGF) appears to function as a wound hormone. PDGF is normally released at sites of trauma during platelet-aggregation, where it acts as a potent chemoattractant for connective tissue cells. In concert with other peptide growth factors, PDGF stimulates the division of these cells. In vivo studies indicate that addition of PDGF to wounds can accelerate the rate of new tissue formation in both normal as well as wound repair impaired animals. In order to better characterize the role of PDGF in wound repair we have investigated animals models with decreased levels of circulating platelets, such as animals undergoing chemotherapy with adriamycin. In these studies we showed that treatment of animals with adriamycan prior to wounding caused an 80% reduction in the amount of new tissue formed at the wound site while treatment during the time of repair had no inhibitory effect. This indicates that adriamycin impairs wound repair via an indirect mechanism, possible by decreasing the levels of PDGF and other wound signals produced immediately at the time of wounding which are essential for the initiation of the repair process.

Related studies indicate that there are specific factors for each cell type which participates in the repair process. We are presently involved in the characterization and isolation of chemotactic factors for endothelial cells and skin epidermal cells which are present at wound sites. These factors might be used to augment the levels normally present in various wounds and accelerate the rate of repair.

LABORATORY OF ORAL MEDICINE

Summary Report

The Laboratory of Oral Medicine studies the etiology and pathogenesis of both systemic diseases and diseases of the soft tissue of the oral cavity. Emphasis is on: (1) viral infections such as herpes simplex virus; (2) endocrine diseases, especially virus-induced diabetes mellitus; (3) autoimmune disorders; and (4) ulcerative and proliferative lesions of the oral cavity. The program is disease oriented and highly interdisciplinary. The Laboratory is made up of investigators who are trained in a variety of disciplines including virology, immunology, pathology, molecular biology, and clinical medicine and dentistry.

Over the last year, in-depth studies have continued on the projects discussed in previous Annual Reports. Good progress has been made in most areas and a recombinant DNA vaccine against herpes simplex virus type 1 (HSV-1) has been made and shown to be effective in experimental animals in preventing both lethality and the development of latency.

Since last year, several members of the Laboratory have left: Dr. John Hooks, who worked on interferon; Dr. Edouard Cantin, who was working on HSV; and Dr. Takashi Onodera, who headed the Laboratory's efforts in virus-induced autoimmunity. As a result of Dr. Hooks' departure, the project on interferon was terminated. Dr. Cantin, who is now at the City of Hope, is still attempting to express the B_2 gene of HSV-1 (i.e., glycoprotein B_2) in E. coli. This continues as a collaborative project with LOM. Dr. Takashi Onodera's work on virus-induced autoimmunity has been taken over by other members of the Laboratory. Because of the direction of ongoing projects in the Laboratory, we have decided to fill the vacant slots with people who have training in molecular biology and protein purification.

The members of the Laboratory continue to work well with each other, and there are many collaborative projects. As in past years, the office staff, the tissue culture laboratory, and the pathology unit are functioning very smoothly.

Since last year, a number of new techniques were introduced into the Laboratory and existing ones modified. Specific techniques include: (1) cloning in vectors capable of growth both in procaryotic and eucaryotic cells (shuttle vectors); (2) establishment of a eucaryotic transfection assay system to detect activated oncogenes; (3) development of microneutralization assays for HSV-1, HSV-2, and vaccinia virus to detect neutralizing antibodies; (4) refinement of DNA sequencing methods; (5) preparation and testing of monoclonal anti-idiotypic antibodies; (6) transformation of human B lymphocytes by Epstein-Barr virus and the production of monoclonal autoantibodies by these transformed cells; (7) establishment of T-cell lines by growth in the presence of T-cell growth factor; (8) immunocytochemistry using electron microscopy; and (9) the avidin-biotin immunoperoxidase technique has been further refined and is now being applied to ultrastructural studies.

Past collaborations continue and a number of new ones have been initiated. Active collaboration projects include: (1) construction and testing the efficacy of vaccinia virus recombinants expressing HSV-1 glycoprotein D as a vaccine against HSV infections (Laboratory of Viral Diseases, NIAID); (2) construction and testing of recombinant vaccine vectors which express the glycoprotein B gene of HSV-1 (City of Hope Medical Center, Duarte, California); (3) attempts to reactivate HSV in the skin by use of UV light (Laboratory of Clinical Investigation, NIAID); (4) studies on the state of the viral genome during latency (Wistar Institute and the University of Pennsylvania); (5) binding of monoclonal antibodies to tumor cells (Department of Microbiology and Immunology, Loma Linda University); (6) studies on tumors of the head and neck (Walter Reed Army Medical Center); (7) development of hybridomas against chondronectin (Laboratory of Developmental Biology and Anomalies, NIDR); (8) Coxsackie B4 virus-induced polymyositis (Arthritis and Rheumatism Branch, NIADDK); (9) development of monoclonal antibodies against osteonectin (Mineralized Tissue Research Branch, NIDR); (10) development of monoclonal antibodies against bone-induction protein (Mineralized Tissue Research Branch, NIDR); (11) analysis of antigenic variants of Coxsackie B4 virus with differences in biological activity (Virginia Commonwealth University); (12) development of monoclonal antibodies against transducin (Metabolic Diseases Branch, NIADDK); (13) long-term prospective study on newly diagnosed insulin-dependent diabetes (Mt. Sinai Medical Center, New York City); (14) studies on autoantibodies in the sera of patients undergoing pancreas transplantation (University of Minnesota); (15) studies on autoantibodies in the sera of patients with congenital rubella (Mt. Sinai Medical Center, New York City); (16) virus-induced diabetes in non-human primates (Infectious Disease Branch, NINCDS); (17) the association of the diabetes (db) gene with the phenotypic expression of islet cell antibodies in mice (The Jackson Laboratory, Bar Harbor, Maine); (18) rubella virus-induced diabetes in hamsters (Mt. Sinai Medical Center, New York City); (19) antibody to islet cell antigens from patients with Vacor-induced diabetes (Chonnam University Medical Center, Kwangju, Korea); and (20) slow viruses and diabetes (Laboratory of Central Nervous System Studies, NINCDS).

Some of our more important findings since last year's Annual Report are summarized below:

1. We have been using the mouse model developed in the Laboratory several years ago to test the efficacy of a new prototype vaccine against herpes simplex virus. A recombinant vaccinia virus which expresses the glycoprotein D of HSV-1 has been genetically engineered by colleagues at NIAID. To determine whether the recombinant virus would induce protective immunity, Balb/c mice were vaccinated with the recombinant vaccinia by three different routes: intraperitoneal, foot pad or the base of the tail. All vaccinated mice produced neutralizing antibodies against HSV-1. When challenged with a predetermined lethal dose of HSV-1, over 90% of the vaccinated mice were protected, whereas control animals or animals vaccinated with wild type vaccinia or another recombinant expressing the surface antigen of hepatitis B were not protected.

To determine the efficacy of vaccination with the vaccinia recombinant on the prevention of HSV latency, mice vaccinated by the tail route were challenged with HSV-1 by the labial route at a dose sufficient to establish a latent infection in 90-100% of control mice. Our experiments showed that the recombinant virus was 70% effective in preventing the development of latency. This is the first demonstration of the effectiveness of a genetically engineered vaccine to prevent the establishment of an HSV latent infection.

2. During the last year we have continued our studies of the relationships at the DNA sequence level between herpes simplex virus type 1 (HSV-1) and c-Ha-ras1, the oncogene isolated from the human bladder carcinoma cell line T24. This oncogene is a homologue of v-Ha-ras, the retroviral oncogene of Harvey Murine Sarcoma Virus. We have mapped by restriction enzyme analyses the regions that are related in the two DNAs and have found that they occur in non-translated segments of the respective genomes. However, these segments play an important role in gene expression. The c-Ha-ras1 segment is essential for transformation, since its deletion abolishes the transforming activity of the oncogene in DNA-mediated gene transfer experiments that use NIH/3T3 as recipient cells. Its related segment in the HSV-1 genome is located within a DNA segment that has transcriptional enhancer properties for one of the viral immediate-early (IE) genes.

In an attempt to determine the possible biological consequences of these sequence relationships, we have constructed recombinant DNA plasmid chimeras in which the oncogene segment related to HSV-1 DNA was replaced by its related HSV-1 enhancer fragment. These plasmids were used to transfect NIH/3T3 cells. The chimeras were as efficient in transformation as the unaltered c-Ha-ras1 oncogene, whereas replacement of the oncogene segment by an irrelevant DNA fragment from the plasmid pBR322 abolished its transforming activity. Similar constructs were made using the non-transforming cellular homologue of c-Ha-ras1, and, as expected, they did not transform NIH/3T3 cells.

The implications of these experiments in light of the hit-and-run nature of HSV transformation are attractive, albeit speculative at the moment. Sequence relatedness may provide sites for illegitimate recombination events to occur between HSV DNA and the normal homologue of c-Ha-ras1. Insertion of the HSV IE gene enhancer may activate transcription of the cellular p21 protein and inactivate viral gene expression, allowing for the unmasking of seldom-expressed non-lytic viral functions. One of these functions may be transformation. We are currently testing the salient features of this hypothesis.

3. Previous work in this Laboratory identified sequences in the mouse and human genomes related to the HSV DNA. Two mouse clones (TGL19 and TGL35) homologous to the terminal repetitions of the HSV-1 genome were isolated from a mouse trigeminal ganglion DNA library. To characterize the degree of relatedness between the mouse and HSV-1 DNA, we determined the nucleotide sequence of the regions involved in the homologies. Both murine clones have

greater than an 80% base match with reiteration II of the SmaI A fragment of HSV-1 DNA showing that the homology is specific and is not a spurious interaction among GC-rich DNA. Computer analysis indicated that these cellular and viral clones are related to immunoglobulin (Ig) class-switch sequences. The relationship with the murine genome indicates that a DNA family with features like an Ig switch sequence may exist. This observation is now being studied in depth.

4. During the past year a transfection system to detect activated oncogenes was initiated. The system employs both NIH/3T3 cells and secondary rat embryo fibroblasts to detect the largest possible spectrum of oncogenes. The system is validated with both cloned myc and ras oncogenes as well as tumor cell line DNA. Recombinant DNA constructs, coupling HSV sequences with the coding sequences of the T24 oncogene (c-Ha-ras1), transformed NIH/3T3 cells. The number of foci induced by the constructs was roughly equivalent to that induced by the intact T24 oncogene, but three orders of magnitude greater than the T24 coding sequence alone. The transfection system is now being used in a number of different studies in the Laboratory.

5. Further progress has been made on the nature of the autoantigens with which certain human monoclonal antibodies react. By fusing peripheral leukocytes from a patient with insulin-dependent diabetes with mouse myeloma cells, a heterohybridoma was isolated that for over one year has secreted a human monoclonal autoantibody, designated MOR-h1. This antibody reacts with antigens in several endocrine organs including the pituitary, thyroid, stomach, and pancreas. By double immunofluorescence, MOR-h1 was found to react specifically with growth hormone (GH)-containing cells in the anterior pituitary and, by ELISA, MOR-h1 was shown to react with both natural and biosynthetic GH. Absorption experiments revealed that GH could remove the capacity of MOR-h1 to react not only with cells in the anterior pituitary, but also with cells in the thyroid, stomach and pancreas. The demonstration with hyperimmune serum that these organs do not contain GH indicated that MOR-h1 was reacting with a different molecule(s) in these organs. By passing extracts of pituitary, thyroid and stomach through an MOR-h1 affinity column and analyzing the eluted antigens by SDS-PAGE, a 35K polypeptide was isolated from each of these organs. In addition, a 21.5K polypeptide with an electrophoretic mobility identical to purified human GH was isolated from the pituitary, but not the other organs. It is concluded that MOR-h1 reacts with a 35K polypeptide present in the pituitary, thyroid, and stomach and that this antibody also recognizes a determinant on GH.

6. Isolation and characterization of the antigens recognized by human monoclonal autoantibodies are being attempted with the goal of identifying the antigens or those characteristics of the antigens that might be useful diagnostically and prognostically. Thus far, we have examined six such autoantibodies (MOR-h1, MOR-h2, MOR-h3, MOR-h7, WK17G9, and CN33H8). All of these are multiple organ-reactive and all are of the IgM class of immunoglobulins.

Using immunoaffinity columns prepared with the purified monoclonal autoantibodies, antigens have been isolated from one or more of the target organs. These antigens have been characterized by SDS-PAGE, ELISA, and RIA.

As already indicated, MOR-h1 has been found to react with human growth hormone in the pituitary as well as a 35,000 m.w. polypeptide in the pituitary, stomach, and thyroid. Polypeptides of approximately 10,000 m.w. have been isolated from human gastric mucosa using MOR-h2 and MOR-h7 columns. MOR-h3 column eluates of gastric mucosa extracts contain a protein of about 25,000 m.w. Using WK17G9, which reacts with an antigen on the surface of pancreatic beta cells, a 10,000 m.w. protein has been isolated from rat insulinoma cells. Thus far, all of these putative autoantigens (except for MOR-h1) have been isolated in only microgram quantities. Attempts are being made to increase the yield of these proteins so that a more complete serological and biochemical analysis can be done.

7. Studies over the last year have shown that human lymphocytes transformed by Epstein-Barr virus (EBV) make autoantibodies. In brief, peripheral blood lymphocytes (PBL) of normal individuals and patients with autoimmune abnormalities were transformed in vitro by EBV and tested for autoantibodies to normal tissues. High proportion of EBV-transformed B-cell cultures were found to produce autoantibodies that reacted with normal tissues. Selected EBV-transformed B-cell cultures making autoantibodies were cloned and the monoclonal autoantibodies were studied for their reactivity with a panel of tissue sections from different organs. All the EBV-induced monoclonal autoantibodies were found to react with antigens in more than one organ (e.g., thyroid, pancreas, stomach, smooth muscle, nerve). The present results make it clear that a virus can trigger the production of autoantibodies through its proliferative effect on B lymphocytes without the virus infecting and altering target cells to which the autoantibody is directed.

8. Using hybridoma technology, we have shown that lymphocytes from normal mice can make autoantibodies. Many of these antibodies reacted with multiple organs (MOR type) and gave reactivity patterns similar to those observed with MOR antibodies isolated from animals and patients with autoimmune disorders. These observations argue that lymphocytes that make MOR antibodies are common and part of the normal B-cell repertoire. The current observation also makes it clear that hybridomas prepared from animals with autoimmune disease may not necessarily secrete autoantibodies specific to that disease. Furthermore, these studies indicate that cells capable of making autoantibodies are present, but silent, in normal animals and can at times be triggered by environmental factors to produce autoantibodies in the absence of specific stimulation by autoantigens.

9. The studies initiated last year with Mengovirus have been extended. In mice, Mengovirus produces a fatal encephalitis. Plaque purification of the virus resulted in the isolation of a clone (Mengo-2T), which in addition to encephalitis caused diabetes. Microscopic examination of pancreases from infected mice revealed necrosis in the islets of Langerhans and infiltration

of inflammatory cells. By immunofluorescence viral antigens were found in the islets, and radioimmunoassays demonstrated a substantial decrease in pancreatic immunoreactive insulin. Studies on susceptibility among inbred strains of mice showed that whereas the D variant of encephalomyocarditis virus caused diabetes only in SJL/J mice, Mengo-2T caused diabetes in strains of mice resistant to encephalomyocarditis-induced diabetes (i.e., CBA/J, C3H/HeJ, CE/J, AKR/J, C57BL/6J). The ability of Mengo-2T to induce diabetes in encephalomyocarditis-resistant mice was found to be due to the greater capacity of Mengo-2T as compared to the D variant of encephalomyocarditis virus to replicate in and destroy the islets of these animals. Although Mengo-2T and the D variant of encephalomyocarditis virus are antigenically indistinguishable by hyperimmune sera, our studies show that these viruses have different host ranges and tissue tropisms.

10. "Molecular mimicry" may be one of the mechanisms by which viruses can trigger an autoimmune response. The idea is that antibodies raised against certain viral antigens may cross-react with normal host cell antigens. To study this possibility, we screened several hundred monoclonal antiviral antibodies for their cross-reactivity with normal tissues. Approximately 3.5% of the monoclonal antiviral antibodies tested reacted with perfectly normal tissues and many reacted with antigens in more than one organ. These interesting observations are now being studied in greater depth.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00080-11 LOM

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Yoon, Ji-Won	Microbiologist	LOM, NIDR
Garzelli, Carlo	Visiting Fellow	LOM, NIDR
Kim, Chang J.	Guest Worker	LOM, NIDR
Onodera, Takashi	Expert	LOM, NIDR
Saegusa, Junzo	Visiting Fellow	LOM, NIDR
Srinivasappa, Javaraiah	Visiting Fellow	LOM, NIDR
Taub, Floyd E.	Sr. Staff Fellow	LOM, NIDR
Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
11.27	5.91	5.36

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The laboratory continues to search for viruses that can produce diabetes in animal and man. Extensive studies over the last several years have shown that the development of encephalomyocarditis (EMC) virus-induced diabetes depends on the genetic background of the host and the genetic makeup of the virus. Recently, we found that Mengovirus 2T can infect and destroy pancreatic beta cells and produce a diabetes-like syndrome in mice. More detailed studies over the last year have revealed that whereas EMC virus causes diabetes in only certain inbred strains of mice, Mengovirus 2T caused diabetes in strains of mice resistant to EMC virus-induced diabetes. The ability of Mengovirus-2T to induce diabetes in EMC-resistant mice was found to be due to the greater capacity of Mengovirus 2T as compared to EMC virus to replicate in and destroy the islets of these animals. In addition, we found that Cotton rats develop diabetes when infected with a lethal dose of Mengovirus 2T. We also have preliminary evidence that monkeys develop abnormal glucose tolerance tests when infected with certain viruses and subdiabetogenic doses of streptozotocin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00123-11 LOM

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Herpes Simplex Virus and Other Oral Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Puga, Alvaro	Expert	LOM, NIDR
Brayton, Peter R.	Staff Fellow	LOM, NIDR
Cremer, Kenneth J.	Sr. Staff Fellow	LOM, NIDR
Eskinazi, Daniel	Visiting Scientist	LOM, NIDR
Gomez, Jaime	Vistiing Fellow	LOM, NIDR
Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Viral Disease, DIAID (B. Moss)
City of Hope Medical Center, Duarte, CA (E. Cantin)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
9.25	5.33	3.92

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

We have continued to investigate the molecular events leading to the establishment of herpes simplex virus (HSV) latent infections in the nervous system of experimentally inoculated mice and are determining the possible role of herpes simplex virus in neoplastic transformation. Regions of homology between the terminal repetitions of the HSV genome and the genomes of mice and humans have been identified and we have determined the nucleotide sequence of the viral and cellular DNA involved in these homologies. One of these regions is localized in the terminus of the HSV genome and shows strong homology with mouse DNA sequence very similar to immunoglobulin heavy chain genes involved in class-switch recombination. Another region of homology was found between an intergenic region of the immediate-early genes of HSV that has transcriptional enhancer activity and the regulatory region of the c-Ha-ras1 oncogene isolated from the T24 human bladder carcinoma cell line. Deletion of the regulatory region in the oncogene led to the loss of its transforming activity, but its replacement by the HSV enhancer sequences in recombinant plasmid chimeras fully restored the transformation capacity of the c-Ha-ras1 oncogene.

A recombinant vaccinia virus which expresses glycoprotein D of HSV-1 has been tested for its efficacy as a live vaccine to prevent primary infections with HSV. Mice vaccinated with the recombinant virus developed neutralizing antibodies against HSV-1 and the vaccine protected mice against a lethal intraperitoneal challenge with HSV-1. The recombinant virus also prevented the establishment of a latent HSV infection in many of the experimentally challenged mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00309-04 LOM

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Autoimmunity and Hybridomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Prabhakar, B.	Sr.Staff Fellow	LOM, NIDR
McClintock, P.R.	Expert	LOM, NIDR
Drell, D.W.	Staff Fellow	LOM, NIDR
Essani, K.	Visiting Fellow	LOM, NIDR
Garzelli, C.	Visiting Fellow	LOM, NIDR
Ray, U.	Visiting Associate	LOM, NIDR
Saegusa, J.	Visiting Fellow	LOM, NIDR
Satoh, J.	Visiting Fellow	LOM, NIDR
Uchigata, Y.	Visiting Fellow	LOM, NIDR
Taub, F.E.	Sr. Staff Fellow	LOM, NIDR
Notkins, A.L.	Medical Director	LOM, NIDR

COOPERATING UNITS *(if any)*

Mt. Sinai School of Medicine (F. Ginsberg-Fellner)

LAB/BRANCH Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
15.23	8.16	7.07

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have isolated human monoclonal autoantibodies that each react with antigens in a variety of organs and are termed multiple organ reactive (MOR) antibodies. Using MOR immunoaffinity columns we have attempted to isolate and characterize the autoantigens in human tissues. We are attempting to determine whether diagnostically or prognostically useful screening procedures can be devised for the antigens and/or anti-idiotypic antibodies that react with MOR antibodies.

We have found that lymphocytes capable of producing MOR antibodies are a normal part of the B cell repertoire. Using both standard fusion techniques and EBV transformation, cell lines secreting monoclonal MOR autoantibodies were obtained using lymphocytes from normal individuals. Thus, lymphocytes capable of making autoantibodies are present, but silent, under normal conditions but can at times be triggered by environmental factors to produce autoantibodies.

CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH SUMMARY STATEMENT

The Clinical Investigations and Patient Care Branch functions as the nucleus of the Institute's clinical activities. As such it has multiple major and varied responsibilities. These include the following: (1) to conduct high quality, clinically oriented dental research programs; (2) to render clinical care to specified patients of the Clinical Center; (3) to offer consultation on oral and dental problems to other institutes; (4) to encourage and to provide support, consultation and facilities for clinical research activities of other Branches and Laboratories within the Institute; and (5) to sponsor a training program, the Clinical Dental Staff Fellowship, aimed at developing academic and research oriented dental clinicians.

The past year has been marked by the solidification of programs begun with the reorganization of the Branch in 1982 and considerable progress towards our stated goals. A high level of effort, cooperation, understanding and motivation by all Branch personnel have enabled us to continue to develop our investigative programs and to broaden our already active and valued presence within the Clinical Center.

Patient Care Section

The Patient Care Section conducts the daily operation of the NIDR clinic and as such is the focus of clinical oral and dental health concerns at NIH. The Section provides a wide range of diagnostic consultative services to NIH clinical care programs. The section has continued to become more involved with the medical activities of the various Institutes and of the Clinical Center. Staff dentists and dental hygienists routinely participate in medical rounds and patient care discussions thus integrating oral health care concerns to total patient management. The Section is also responsible for providing clinical training and development for the Dental Staff Fellows as well as support for clinical research activities.

The Patient Care Section continued affiliations with the Baltimore College of Dental Surgery, University of Maryland and Montgomery College. These collaborations provided graduate endodontic, senior dental hygiene, dental assistant, and dental laboratory students an opportunity to participate in alternative practice settings beyond those offered in the school core curriculum. The arrangements provided our staff with academic clinical dental teaching responsibilities and offered a professional development opportunity. The Section began participation in the NIH Clinical Electives Program. Two senior dental students have been selected for the 1984-1985 academic year. They are to serve in an eight week program which emphasizes the provision of dental care to medically compromised patients in a hospital environment. These two students will also be encouraged to visit the various

intramural research laboratories within the NIDR and become familiar with their activities.

The Section's staff has been deeply involved in the further development of the Dental Staff Fellow program. Formal training presentations include regularly scheduled rounds and a Fellowship lecture series. The lecture series has brought in speakers from outside the NIH, from other NIH institutes as well as from the various Laboratories and Branches of the Intramural Research Program, NIDR. Also Journal Review Seminars are held giving the staff an opportunity to present and discuss interesting articles from the dental and basic science literature. Many of the Patient Care Section staff members have been active in both clinical and laboratory research projects. These projects include the following: (1) description and management of patients with unusual oral manifestations or complications of systemic disease; (2) the use of occlusal fissure sealants as a possible vehicle for slow release of fluoride; (3) epidemiological and clinical parameters of jaw lesions in Burkitt's lymphoma in the American population; (4) oral and facial development in patients with precocious puberty; (5) development of treatment protocols for prevention and management of sequelae to oncology therapy; and (6) development of immunocytochemical methods to aid in the diagnosis of malignancies.

Clinical Investigations Section

This section was created 2 years ago expressly to establish a high quality clinical-problem oriented, dental research program. The general theme of our investigative efforts is understanding the regulation of membrane-associated cellular processes. Specifically this is manifested in three problem areas: (1) the control and mechanism of secretory events in salivary gland cells; (2) the role of endogenous antioxidant systems in cell protection; (3) the diagnosis and treatment of salivary gland secretory dysfunction and management of associated oral disorders.

Considerable effort is directed at understanding specific biochemical mechanisms involved in the formation of saliva. It is well accepted that saliva has a critical role in the defense, and functional maintenance, of all oral tissues. Saliva contains water and electrolytes, derived from serum, and specific exocrine proteins synthesized by glandular epithelial cells. Furthermore salivary glands are widely studied models of exocrine secretory processes. Our investigations utilize rat salivary glands with both in vitro and in vivo experimental systems and employ as experimental paradigms (1) age-associated alterations and (2) therapy-induced gland dysfunctions.

One main thrust of study is directed towards understanding secretory events related to water and electrolyte movement. These responses are primarily under α_1-adrenergic and muscarinic-cholinergic control. Utilizing a model of aging, we have previously shown that at least two distinct transduction mechanisms are likely involved in α_1-adrenergic, Ca^{2+}-mediated secretory events in the rat parotid acinar cell. In addition we have been able to dissociate clearly α_1-adrenergic and muscarinic-cholinergic

responses. Because these receptors elicit their effects, in part, due to major changes in membrane permeability, we have initiated studies aimed at clarifying ion fluxes associated with early events in secretion.

The approach taken was to develop a transport model, using parotid basolateral membrane vesicles. A similar model has been employed in studies of kidney tubule and intestinal cell transport. We have demonstrated that vesicles prepared with this tissue exhibit a Na^+-dependent α-aminoisobutyrate transport system (system A) with characteristics comparable to those seen with intact cells. Utilizing such vesicles, we next studied Ca^{2+}-transport mechanisms. Our experiments have shown that parotid basolateral membranes contain both an ATP-dependent Ca^{2+} transport system and a Na^+/Ca^{2+} exchange mechanism.

In related studies, employing intact cells in vitro, we have continued to examine receptor regulation of ion fluxes. Studies with parotid cells have examined the possible role of Na^+ influx in either the initial or maintenance phases of α_1-adrenergic elicited secretions. Our data suggest Na^+ influx may be particularly important in prolonging the high levels of intracellular free Ca^{2+} required for maintaining secretion. Another group of experiments has begun a detailed characterization of Ca^{2+} mobilization by rat submandibular cells after adrenergic and muscarinic-cholinergic stimuli. This is of particular interest to us because previously we have shown that changes in Ca^{2+} mobilization are associated with a marked, and specific, inhibition of protein synthesis in these cells. During this reporting period we have studied the time-kinetics of Ca^{2+} uptake and efflux and the role of specific autonomic receptors in regulating these events (Ca^{2+} movement, protein synthesis inhibition). Our data are consistent with an hypothesis that rapid mobilization of Ca^{2+} pools (likely sequestered in microsomal membranes) for secretory events, results in the inhibition of a Ca^{2+}-requiring step in protein synthesis.

The other major constituents of saliva are exocrine proteins, including several glycoproteins with important functional roles in oral health maintenance. Previously we have reported that β-adrenoreceptor stimulation elicits a marked (2-3 fold) enhancement of N-linked protein glycosylation and this phenomenon is mediated by cyclic AMP. Considerable effort during this reporting period has been directed at beginning to unravel the mechanistic steps which lead to this response. We have shown that in intact cells, the β-adrenergic agonist isoproterenol causes a marked enhancement (> 100%) in the formation of the important saccharide-lipid intermediates mannose-PP-dolichol and oligosaccharide-PP-dolichol. Furthermore β-adrenergic stimulation increases turnover of the oligosaccharide-PP-dolichol pool. These events are catalyzed by specific enzymes and we have focused attention on three saccharide-lipid transferases considered to be "key" steps. Our studies have employed microsomal membrane preparations from control and isoproterenol-stimulated cells, and shown activation of enzymic action after neurotransmitter treatment. We observed that N-acetylglucosaminyl -1-phosphate transferase, mannose-P-dolichol synthase and glucose-P-dolichol synthase were each increased about 50% after agonist exposure. In separate,

but related experiments, we have demonstrated that β -adrenoreceptor stimulation also increases the rate of high mannose oligosaccharide processing to complex oligosaccharide forms.

Therapy-associated salivary gland dysfunction is a commonly encountered clinical problem. We have been engaged in studies using rat salivary gland models, aimed at understanding how two frequent therapeutic regimens alter salivary secretion. The first examines how phenothiazines (a category of often-prescribed psychotropic drugs) inhibit salivary secretion. During this reporting period we have shown that chlorpromazine (a representative drug in this class) can block muscarinic-cholinergic but not β -adrenergic elicited secretory responses. This blockade is not related apparently to intracellular effects of the drug (such as interference with calmodulin-mediated actions) but rather is induced by effects of the drug at a receptor level. The second category of therapy-associated perturbations studied is x-irradiation-induced gland alterations. Our work has shown that while acute, single high dose radiation (2000R) markedly inhibits in vivo salivary secretion within three days, receptor-regulated cell function in vitro is unaffected. This suggests that acinar cells may not be the primary target of x-ray damage. In the human clinical situation however chronic irradiation damage is a more relevant concern. Accordingly, we have developed a chronic radiation model with the rat which exhibits dose-dependent x-ray effects on parotid secretion.

The second principal problem area that is studied by the Clinical Investigations Section is the role of endogenous antioxidant systems in mammalian cell protection. This is particularly of relevance regarding mechanisms of cellular damage following ionizing radiation. Another important physiological purpose for such antioxidant systems is in the modulation of immune cell function. Indeed by utilizing lymphocyte models we have previously suggested a central role for the membrane-bound amino acid transport enzyme, γ -glutamyl transpeptidase, and a disulfide reductase, in antioxidant defense mechanisms . It appeared that γ-glutamyltranspeptidase activity was related to cleavage of endogenous disulfides and thus to the generation of protective thiol compounds. During this reporting period we have continued efforts to clarify regulatory and mechanistic aspects of this process. Using specific inhibitors of enzymes in the γ -glutamyl cycle, we have shown a clear requirement for glutathione synthesis for disulfide cleaving ability. No such requirement for γ -glutamyltranspeptidase activity was seen. It thus seemed that the simple availability of glutathione was sufficient for the development of γ-glutamyl transpeptidase activity. Studies were then designed to characterize the induction mechanism for these antioxidant activities. The data indicate that any change in the redox potential of the cell can lead to activation of disulfide cleaving and γ -glutamyl transpeptidase activities.

The final major problem area studied by our section focuses on the clinical manifestations of altered secretory processes. During the past year considerable effort has been directed towards establishing a proper diagnostic approach for the evaluation of salivary gland disorders and towards developing treatment protocols aimed at improving salivary gland performance and

associated oral functional disturbances. We have received many referred patients from other Institutes as well as from outside the NIH, through the Dry Mouth Evaluation Clinic. Salivary gland perfomance and diagnoses have been determined by: (1) the collection of unstimulated and stimulated parotid and submandibular/sublingual salivas; (2) quantitative analysis of secretory components of these salivas; (3) ^{99m}Tc salivary scintigraphy and (4) lip biopsy, along with various general systemic diagnostic procedures. The usefulness of these procedures as diagnostic predictors has been examined. We have also been able to define several catagories of salivary gland dysfunction, and based on gland response, been able to define a patient group which would likely respond to drug manipulation to enhance salivary secretory performance. We began an initial therapeutic protocol which utilizes oral administration of the parasympathomimetic agent, pilocarpine, to improve salivary function in "dry mouth patients." Results obtained thus far have shown that considerable improvement in salivary performance can be obtained.

Related studies of oral functions associated with salivary performance have concentrated on (1) gustatory performance and (2) oro-pharyngeal swallowing. Gustatory studies of certain xerostomic patients have demonstrated that individuals may be without salivary gland function (no major gland secretions; minor gland biopsies showing no functional gland parenchyma) yet be free of taste complaints and perform within normal limits on threshold and suprathreshold tests of taste performance. Such results call into question the purported role of saliva constituents in the specific maintenance of the gustatory apparatus and suggest that the primary role of saliva is in the dissolution and presentation of tastants. Considerable effort has also focused on measurement issues in analyzing and communicating the results of psychophysical tests of taste performance. In particular, a new percentile-based scale has been developed to allow statistically valid comparisons of individual patient results to be made with broadly based, normative population data. Still other studies, employing patients with partial dysgeusia, have emphasized the functional importance of taste receptors not located on the dorsal-lingual surface of the tongue in normal gustatory function.

Patients with oral dryness often complain of swallowing difficulties. In order to evaluate more accurately the oropharyngeal phase of swallowing, we have built on our past experience with physical evaluations of oral-motor performance and with our use of ultrasonography, to evaluate tongue function non-invasively. We have employed a convenient, cranial nerve-based, physical examination procedure to evaluate oral muscular function in patients and developed a clinically useful way to summarize and transmit these results. Using real time ultrasound we have succeeded in defining specific stages of oropharygeal swallow and have been able to characterize the functional relationships between the tongue, larynx and hyoid in a group of normal individuals. Comparative study of patients with frank dysphagia due to cranial nerve damage has allowed a clearer picture of alterations in the swallowing mechanism. This framework has improved our ability to separate compensatory changes in tongue function, such as may occur during normal aging, from pathologic processes.

PUBLICATIONS FISCAL YEAR 1984

CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00212-08 CI

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Taste and Its Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Weiffenbach, J.M., Psychologist, CIPCB, NIDR
Baum, B.J., Dental Officer, CIPCB, NIDR
Fox, P.C., Dental Officer, CIPCB, NIDR

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda Maryland 20205

TOTAL MAN-YEARS .80	PROFESSIONAL .80	OTHER

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This project seeks to elucidate the mechanisms by which oral sensory and perceptual experience is generated. Since separate measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, responses to each of the four basic taste qualities are quantified in terms of (detection) thresholds and judgments of the intensity and pleasantness of taste stimulation at highly, more commonly-encountered levels of strength. Functional variation is important to the understanding of underlying mechanism. Measures of variation in taste perception are obtained through objective evaluation of age-associated and therapeutically induced changes and of oral sensory disturbances ocurring in association with systemic disease, salivary gland dysfunction or as an isolated complaint. Assessments of olfaction and of oral tactual sensitivity are obtained when they can contribute to an understanding of oral sensory function in relation to the complex stimuli encountered in everyday life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00320-04 CI

PERIOD COVERED

October 1, 1983-September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Survey of Jaw Lesions in Burkitt's Lymphoma in the NIH Population

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Donahue, Agnes H.	Senior Staff Fellow	CIPCB,	NIDR
Others:	Sariban, Eric	Clinical Associate	PB,	NCI
	McGrath, Ian T.	Senior Medical Staff	PB,	NCI

COOPERATING UNITS *(if any)*

Pediatric Branch, NCI, NIH

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.05	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

  ☒ (a1) Minors

  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The epidemiological and clinical parameters of jaw lesions in Burkitt's lymphoma in the American population are being studied retrospectively. Techniques include chart review of demographic and clinical data of all patients admitted to the NIH Clinical Center with a confirmed diagnosis of Burkitt's lymphoma who have primary or metastatic jaw disease. The National Cancer Institute is a cooperating unit in the study and is included in their project number Z01 CM 06815.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00332-03 CI

PERIOD COVERED

October 1,1983- September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Investigations and Case Reports

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roberts, Michael W.	Chief, Patient Care Section	CIPCB, NIDR
Others:	Butler, Donald P.	Senior Staff Dentist	CIPCB, NIDR
	Drum, Margaret A.	Clinical Staff Dentist	CIPCB, NIDR
	Donahue, Agnes H.	Senior Staff Fellow	CIPCB, NIDR
	Folio, John	Senior Staff Dentist	CIPCB, NIDR
	Martin, Sue E.	Pathologist	CIPCB, NIDR
	Perna, John J.	Dental Staff Fellow	CIPCB, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Pathology and Pediatric Branch, NCI, NIH; Inter-Institute Genetics Program, CC, NIH; Epidemiology Service, CC, NIH; Development and Metabolic Branch, NINCDS, NIH; Georgetown University

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.9	1.6	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews Chart reviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical case studies of unusual interest and clinically related research are being conducted on a variety of dentally related subjects. Research techniques being utilized include chart and literature reviews and evaluation of various therapeutic regimens. Studies are underway in Reiger's syndrome, Albright's hereditary osteodystrophy (AHO), Job's syndrome, acquired immune deficiency syndrome (AIDS), mucopolysaccharidoses, oral health care for oncology patients, head and neck radiation, herpes simplex stomatitis, small-cell osteosarcoma and human papilloma virus DNA in oral lesions.

Others (continued):

van der Ven, Peter F.	Dental Staff Fellow	CIPCB, NIDR
Van Story-Lewis, Patricia	Dental Staff Fellow	CIPCB, NIDR
Wright, William E.	Senior Staff Dentist	CIPCB, NIDR
Rams, Thomas E.	Guest Worker	CIPCB, NIDR
Termine, John D.	Chief, MTRB	MTRB, NIDR
Guckes, Albert D.	Chief, CODC	CC, NIH
Henderson, David K.	Hospital Epidemiologist	CC, NIH
Barton, Norman W.	Asst. Staff Neurologist	DMB, NCI
Constantopoulos, George	Research Biochemist	DMB, NCI
Magyarosy, E.	Visiting Fellow	LP, NCI
Pizzo, Philip A.	Chief, PB	PB, NCI
Broring, Charles L.	Chairman, Pedodontics	Georgetown Univ.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00336-03 CI

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Salivary gland secretory mechanisms during normal and altered functional states

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Baum, B.J., Dental Officer, CIPCB, NIDR

Others:
Takuma, T., Visiting Fellow, CIPCB, NIDR
Helman, Y., Visiting Fellow, CIPCB, NIDR
van der Ven, P., Dental Staff Fellow, CIPCB, NIDR
Fox, P.C., Dental Officer, CIPCB, NIDR
Roth, G.S., Research Chemist, CPB, NIA
Hand, A.R., Dental Officer, LOBP, NIDR

COOPERATING UNITS (if any)
CPB, NIA; LOBP, NIDR

LAB/BRANCH
CIPCB

SECTION
CIS

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda, Maryland 20205

TOTAL MAN-YEARS 4.8	PROFESSIONAL 3.4	OTHER 1.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The health status of the oral cavity is in large part influenced by constitutents of saliva. The principal function of salivary glands is the production of this complex fluid. To study the mechanisms controlling secretion of salivary components we have utilized both in vitro and in vivo animal preparations. As probes of secretory events we have focused our studies primarily on perturbations in neurotransmitter mediated control of gland secretory functions that occur with normal aging. Secondly, we have studied mechanistic events associated with two types of therapy-induced salivary gland dysfunctions; treatment by x-radiation and by phenothiazine. During the present reporting period we have focused efforts on (1) the mechanisms of Ca^{++} mobilization, in intact salivary gland cells and in isolated basolateral plasma membrane vesicles; (2) α_1-adrenergic receptor regulated secretory events; (3) the handling of ^{99m}Tc by salivary cells; (4) development of a model of chronic radiation damage to salivary glands; and (5) characterizing the mechanism of phenothiazine-induced salivary gland dysfunctions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 337-03 CI

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Oral Physiological Processes: Normal Function and Disease Perturbation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*
PI: Fox, P.C., Dental Officer, CIPCB, NIDR
Baum, B.J., Dental Officer, CIPCB, NIDR
van der ven, P., Dental, Staff Fellow, CIPCB, NIDR
Weiffenbach, J., Research Psychologist, CIPCB, NIDR
Martin, S.E., Medical Officer, CIPCB, NIDR
Sonies, B.C., Speech Pathologist, RM, CC
Shawker, T.H., Medical Officer, DR, CC
Hathorn, J., Medical Staff Fellow, POB, NCI; Heft, M., Staff Fellow, LBS, NIA

COOPERATING UNITS *(if any)*
RM, CC; DR, CC; POB, CC; LBS, NIA

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.7	2.2	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This project examines the function of various oral tissues during physiologic aging and in individuals with alterations of normal oral function due to disease or therapeutic procedures. Major efforts have been directed at the evaluation of patients complaining of xerostomia (oral dryness) utilizing the inpatient and outpatient services of the Dry Mouth Evaluation Clinic. Specific diagnostic approaches have been developed to aid in establishing the etiology of salivary gland dysfunction and defining criteria necessary for management decisions. A treatment protocol for selected patients with demonstrable functional gland mass yet inadequate basal salivary performance has been initiated, employing a regimen of oral administration of the parasympathomimetic drug, pilocarpine. In addition, detailed studies of salivary-associated oral complaints (eg taste and oro-pharyngeal swallowing disorders) have commenced. Such studies include evaluation of oral sensorimotor performance across the adult life-span in order to better understand dysfunctional states. Studies on therapy-associated perturbations of oral function have concentrated on the effects of combined chemotherapy for neoplasia on salivary glands and oral soft tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 338 -03 CI

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
The Role of Oxygen Radicals in Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Hoffeld, J.T., USPHS Sr. Dent. Surg., CIPCB, NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

These investigations were conducted to further define the protective extracellullar antioxidative mechanisms of various cell types. Proliferating and metabolically active cells, when incubated in the presence of disulfides, thiols or antioxidants, develop the capacity to cleave extracellular disulfides and show enhanced cell membrane-associate γ-glutamyl transpeptidase (γ-GTP) activity. The thiols released into the medium by these two mechanisms prevent oxidative damage to the cells, a condition permissive for enhanced cell function and proliferation. In all work on this project, cell proliferation, disulfide cleaving activity and γ-GTP activity increased proportionally under conditions of cell stimulation and decreased proportionally under conditions of cell inhibiton. Even the specific inhibitor of the γ-GTP enzyme, Acivicin, led to a proportional diminution of proliferation and disulfide cleaving activity also. Inhibition of glutathione synthesis by buthionine sulfoximine had no effect on development of γ-GTP activity, but inhibited proliferation and disulfide cleaving activity. Furthermore, the inhibition was preventable by the inclusion of 2-mercaptoethanol in the incubation medium. These data suggest the dependence of proliferation and disulfide cleaving activity on synthesis of endogenous glutathione, whereas development of γ-GTP activity can utilize extracelllular glutathione. The agents which induce both disulfide cleaving and γ-GTP activities include certain simple thiols and disulfides, non-thiol antioxidants and electron transfer reagents. These data suggest that any change in the redox potential of the culture medium signals the cell to initiate these antioxidant activities. Further studies of the control mechanisms for development of disulfide cleaving and γ-GTP activities are being conducted using transformed cells lines. Selection of cell lines enriched or diminished for one or both activities is in progress so as to permit better definition of induction and inhibition of each activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00359-02 CI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of A Fissure Sealant as a Vehicle for Slow Release of Fluoride

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roberts, Michael W.	Chief, Patient Care Section	CIPCB, NIDR
Others:	Shern, Roald J.	Dental Officer	CP P, NIDR
	Kennedy, John B.	Biologist	CP P, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.15	0.1	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this laboratory investigation is to evaluate the practicality of using an autopolymerizing occlusal fissure sealant as a vehicle for the slow release of fluoride in the oral cavity. Various amounts of finely powdered sodium fluoride was incorporated into the resin material of an autopolymerizing fissure sealant. The resin was cured and placed into a plastic container containing 5 ml's of distilled water. After 1, 7, 30, 90 and 180 days total fluoride ion release for each test and control specimen was determined using a fluoride ion specific electrode.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00361-02 CI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Application of Immunocytochemistry to the Diagnosis of Malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Martin, Sue	Pathologist	CIPCB,	NIDR
Others:	Magyarosy, E.	Visiting Fellow	LP,	NCI

COOPERATING UNITS *(if any)*

Cytopathology Section, Laboratory of Pathology, NCI, NIH

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The diagnosis of malignant cells in body fluids and fine needle aspirates can be extremely difficult using morphological criteria alone. Using lymphoid cell surface markers we have developed a technique for the immunocytochemical diagnosis of lymphoma in cytological specimens. This methodology is applicable to all types of malignant disease, provided one has suitable cell surface markers. We are currently investigating various markers for their usefullness in the diagnosis of metastatic carcinoma in body cavity fluids and needle aspirates. This work should greatly enhance the power of cytological diagnosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00362-02 CI

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dental Development in Patients with Precocious Puberty

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roberts, Michael W.	Chief, Patient Care Section	CIPCB,	NIDR
Others:	Donahue, Agnes H.	Senior Staff Fellow	CIPCB,	NIDR
	Comite, Florence	Clinical Associate	DEB,	NICHHD
	Pescovitz, Ora H.	Medical Staff Fellow	DEB,	NICHHD
	Loriaux, Lynn D.	Chief, DEB	DEB,	NICHHD
	Hench, Karen B.	Associate Investigator	CC N,	NICHHD
	Li, Shou-Hua	Visiting Scientist	CP P,	NIDR

COOPERATING UNITS *(if any)*

Developmental Endocrinology Branch, NICHD, NIH

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Attempts are being made to determine if there are any correlations between skeletal age, chronological age, tooth eruption and dental root formation in patients with precocious puberty. Oral examinations have been completed on 112 children and panographic radiographs obtained on 97. Using the most accurate available dental growth and development standards comparative studies are being made. These studies will involve both cross sectional and longitudinal evaluations. The NICHHD is a cooperating unit in the study and has assigned the project number Z01 HD 00610.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00372-02 CI

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
N-linked protein glycosylation and β-adrenoreceptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kousvelari, E., Senior Staff Fellow, NIDR, CIPCB

Others: Baum, B.J., Dental Officer, CIPCB, NIDR
Grant, S.R., Research Associate, M.D. Anderson Hospital
Banerjee, D., Visiting Associate, CIPCB, NIDR

COOPERATING UNITS (if any)

Biochemistry and Molecular Biology Department, M.D. Anderson Hospital
and Tumor Institute, Houston, Texas

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.8	2.3	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Saliva contains a broad spectrum of proteins which are known to play an important role in maintaining the integrity of the hard and soft oral tissues. Many of the proteins secreted from the parotid glands are glycoproteins and have their oligosaccharides attached to asparagine residues, so-called N-linked glycoproteins. To study the mechanisms envolved in processing, synthesis and secretion of N-linked secretory proteins, we have utilized in vitro cell preparations from rat parotid glands. We have shown that the potent β-adrenergic agonist (-)-isoproterenol increases $[^3H]$ mannose incorporation into newly synthesized glycoproteins. This effect in the rat parotid acinar cells is mediated by cyclic AMP. During the present reporting period, we have investigated (1) the synthesis of mannosylphosphoryl-dolichol, oligosaccharide-PP-dolichol and the turn over of the oligosaccharide-PP-dolichol pool after β-adrenoreceptor stimulation; (2) the regulation of several "key" enzymatic steps in protein N-glycosylation which may be reasonable sites for the observed enhancement of protein glycosylation seen after (-)-isoproterenol treatment and (3) β-adrenoreceptor effects on oligosaccharide processing.

Summary Report of the Diagnostic Systems Branch

The Diagnostic Systems Branch (DSB) is concerned predominantly with the isolation and identification of factors limiting performance obtainable from noninvasive diagnostic systems. Particular emphasis is directed toward development of x-ray systems designed primarily for dentistry, but the scope is broad enough to include research applicable to a variety of biomedical tasks.

In keeping with DSB's multidisciplinary approach to systems optimization, existing resources are deployed preferentially in collaboration with other agencies having common interests. These include other groups inside NIH such as Biomedical Engineering and Diagnostic Radiology as well as outside representatives from several universities and federally-supported research institutions.

Current activites emphasize three projects:

1) Development and Evaluation of Improved Diagnostic Systems

2) Enhancement of Diagnostic Images

3) Quantitative Assessment of Changes Associated with Myofacial Pain Disfunction Syndrome.

The first two represent extensions of a long-standing commitment to design and production of a clinically useful, computer-based x-ray system having primary application in dentistry. The third was introduced last year and focuses on the use of new noninvasive methods to improve the detectability of small changes in the shape of the mandibular condyle in order to correlate joint damage with observed neuromuscular and occlusal patterns.

System-development research is an extension of that reported in previous years wherein a prototype miniaturized tomosynthetic x-ray system is being fabricated with extramural support, in collaboration with private interests and the National Bureau of Standards. The device is unique in that it contains no moving parts and is capable of producing a video image in near-real time which can be fed back to a computerized x-ray generator to make possible task-specific control of exposure. Such a system has many advantages which facilitate reliable detection and spatial location of tiny changes in the teeth and supporting bone, occuring over relatively long periods.

Specific research accomplishments in this area include demonstration for the first time that an all-electronic radiographic system can produce clinically acceptable images from a tiny scanning x-ray source situated inside the mouth coupled to a video-based image intensifier. These results confirmed earlier computer simulations which suggested that as few as eight projections differing in angular disparity by as little as four degrees are sufficient to yield clinically meaningful tomographic slices of the teeth and supporting bone. This demonstration also provided tangible evidence that the small differences, formally seen only from digital subtractions of conventional radiographs using

non-screen film, can be detected with more reliability and lower exposure, thanks to the relatively high speed and disparity control afforded by this new filmless design. These results are particularly encouraging because the new system also eliminates the need for fabricating custom stints for each different x-ray projection and facilitates direct computer access to radiographic data.

Other research in this area explored the advantages of a micro-focus x-ray source to achieve unprecedented resolution in the resulting images. Preliminary observations show that tiny fractures in the enamel of mandibular central incisors, which previously were considered radiographically undetectable, become readily apparent when such a source is incorported into the design of the prototype scanning x-ray system described above.

Work continues in the area of automated image registration and analysis with particular emphasis on the selection of task-specific measures of image similarity. One particular aspect of this work deals with the use of both linear and nonlinear warping transformations to facilitate mathematically tractable restoration of projective distortions resulting from inadvertant changes in the position of the x-ray detector relative to the source and irradiated tissues. Other related research has focused on the development of an algorithm for estimating objectively the relative projected area and volume of nodular changes (both radiolucent and radiopaque), resulting from digital subtraction of successive radiographs produced using a nearly identical projective geometry. Preliminary data from controlled calibration studies indicate that the algorithm predicts actual size with reasonable precison throughout the range clinical applications tested.

A new aspect of this project involves the role played by a priori information in the maximum performance obtainable from existing diagnostic systems. Of particular concern is the prevalence of the underlying disease processes and its impact on system performance expressed in terms of task sensitivity and specificity. A collaborative effort is underway with Dr. Samuel Zeichner of the radiological staff at the University of Pittsburgh to do a blind evaluation of an existing radiographic data base to determine the degree to which panoramic vs. full-mouth periapical radiographic surveys are useful independently in detecting biopsy-confirmed, intraosseous, nodular lesions of the jaws. It is anticipated that this study will be useful in characterizing the domain of reasonable diagnostic tasks in dentistry as well as for establishing quantitative bases for evaluating the relative merit of two popular radiographic techniques.

A related study being done in collaboration with physicians at Children's Hospital National Medical Center involves the formal development of a logistic model to predict bases for early discharge of patients undergoing intensive care in an effort to optimize the development of limited resources and better contain associated costs. The basic idea is to use Bayesean statistics to predict the failure of essential organs from ICU data. Previous work in this area has resulted in the successful application of a new continuous variable to characterize the severity of disease measured in terms of an ordered set of clinical outcomes.

Other related research involves the formulation and confirmation of a rigorous statistical model which demonstrates the significance of sampling variance and the normal range of variation in x-ray attenuation present in tissues relative to the noise produced from quantum-limited x-ray exposure. For all exposures required of non-screen dental x-ray film, the model predicts that image quality as determined by the total signal-to-noise ratio is likely to be independent of the film speed.

Research continues toward the development of a portable dental fluoroscope. Recent efforts in collaboration with investigators in the Radiation Physics Group, NBS, and USAIDR focus on the application of an intraoral x-ray detector composed of a specially designed, fiber-optic taper which links a conventional phosphor screen to a microchannel plate image intensifier. The basic concept underlying this configuration was suggested by researchers at the University of Oregon more than 20 years ago, but only recent technological advances render such a design practical.

Considerable effort continues to be expended in the clinical application of research tools developed by DSB in earlier years. Perhaps the most widely spread example involves the use of a digital method to compare periapical radiographs obtained from patients suffering from periodontal disease who are undergoing a variety of controlled treatment regimens. The technique involves registration of successively exposed films using a computer-based projection system and subsequent subtraction of stored images after correcting for contrast differences attributable to incidental changes in x-ray exposure and processing chemistry. Controlled clinical studies done in collaboration with several prominent periodontal research teams confirm previous in vitro studies which demonstrated a significant improvement in the detectability of osseous lesions made possible by the use of this technique.

With one exception, recent research pertaining to the enhancement of diagnostic images centers around filtering schemes designed to eliminate unwanted blur in tomosynthetic reconstructions. The basic question to be answered here is whether theoretically elegant but time-consuming iterative techniques produce results sufficiently superior to simpler deconvolution techniques to justify the additional processing required. Recent studies based on controlled observations by dentists show that relatively simple linear deconvolution schemes produce clinical images which cannot be distinguished reliably from those produced using selected techniques which are computationally more extravagant. Related analytical studies have demonstrated that tractable iterative algorithms can be derived from an idealized model which converge relatively rapidly and appear well-behaved in the presence of substantial amounts of uncorrelated noise.

With regard to research relevant to the etiology of myofacial pain disfunction syndrome considerable effort has been expended in the determination of factors limiting the accuracy of estimates of joint destruction as derived from multiple

computerized tomographic (CT) scans. The basic idea is to produce a detailed three-dimensional replica of joint constituents from serial CT slices using an existing algorithm developed by Drs. Jay Udupa and Gabor Herman at the University of Pennsylvania. Data derived from cadaver specimens show that the maximum error in predicting the measured volume of a human condyle using this algorithm is less than 5% irrespective of projection geometry as determined by the posture of the head relative to the gantry of the x-ray machine. The same data show further that the precision of the volume estimate is relatively independent of the value of the targeting parameters and window settings selected. To the extent that these findings can be demonstrated to be applicable to other globular structures such as kidney, spleen, and media-enhanced liver metastases, it is anticipated that this method will find use in a variety of diagnostic applications outside dentistry.

The recent acquisition of a clinical nuclear-magnetic imaging system by the NIH scientific community has not gone unnoticed by DSB staff, and its application to the diagnosis of pathology in the temporal mandibular joint is already being pursued actively in collaboration with investigators in Diagnostic Radiology and BEIB. Recent NMR images produced with the aid of surface coils as well as CT-derived displays appear to have enough resolution to permit unequivocal identification of intracapsular structures. Future plans in this area involve calibrative studies to determine the extent to which known human pathology and results from clinical models can be correlated with CT and NMR observations.

Other research concerned with myofacial pain disorders involve the determination of changes in the relative distribution of occlusal forces integrated between anterior/posterior, and right/left occlusal regions as influenced by the presence of unilateral myofacial pain. This work is part of a comprehensive investigation of myofacial pain being performed in collaboration with Dr. Raymond Dionne of NAB, NIDR.

Approximately three-quarters of the work described above was accomplished within the facilities occupied by DMS staff on the fifth floor of Building 10 at the Bethesda campus. The rest was done at the laboratories of various collaborators.

Dedicated computing resources within DMS were upgraded significantly this year by the replacement of our 16-bit mainframe DEC computer system with a modern 32-bit VAX 11/750 system. The conversion permits the storage of several images simultanously in core which will simplify software development, facilitate simultaneous usage by a larger number of people, and speed up response which is particularly crucial to future plans to operate on multiple images in the spatial-frequency domain. Conversely, these advantages came at the cost of a significant amount of programming effort directed toward conversion of existing programs and data files to a different format, and the need to master and implement a new operating system.

This year marks the end of Dr. Roelf Groenhuis' three year term as a Visiting

Fellow. Dr. Groenhuis was instrumental in research directed toward the design and development of a practical dental tomosynthetic x-ray system. He is being replaced by a colleague from the Netherlands, Dr. Paul van der Stelt, a dentist/Ph.D. with specific research interests in digital radiology.

Publications:

Albert, A., and Ruttimann, U.: Prediction of an Ordered Categorical Variance from Serial Measurements. Accepted for publication in Biometrika.

Groenhuis, R., Ferwerda, H., and ten Bosch, J..: Scattering and Absorption of Turbid Materials Determined from Reflection Measurements. 1: Appl. Opt., 22: 2456, 1983.

Groenhuis, R., ten Bosch, J., and Ferwerda, H.: Scattering and Absorption of Turbid Materials Determined from Reflection Measurements. 2: Measuring Method and Calibration. Appl. Opt., 22: 2463, 1983.

Groenhuis, R., Webber, R., and Ruttimann, R.: Computer Tomosynthesis of Dental Tissues. Oral Surg., Oral Med., Oral Path., Vol. 56, No. 2, pp 206-214, August 1983.

Groenhuis, R., Ruttimann, U., and Webber, R.: A Prototype Digital Tomographic X-ray System for Dental Application. International Symposium on Medical Images and Icons, Arlington, Virginia, July 24-27, 1984.

Grondahl, K., Grondahl, H., and Webber, R.: Influence of Variations in Projection Geometry on Detectability of Periodontal Bone Lesions. Accepted for publication in J. Clin. Periodontology.

Roberts, D., Pettigrew, J., and Udupa, J.: Three-Dimensional Imaging of the Temporomandibular Joint in Vitro and in Vivo. Proc. of Seventh Annual Symposium on Computer Applications in Medical Care, October, 1983, IEEE Computer Society Press, pp 779-782.

Rosling, B., Slots, J., Webber, R., Christersson, L., and Genco, R.: Microbiological and Clinical Effects of Topical Subgingival Antimicrobial Treatment. J. Clin. Periodontology, 10: 487-514, 1983.

Ruttimann, U., Groenhuis, R., and Webber, R.: Computer Tomosynthesis: A Versatile Three-Dimensional Imaging Technique. Proc. of Seventh Annual Symposium on Computer Applications in Medical Care, October 1983, IEEE Computer Society Press, pp 283-786.

Ruttimann, R., Groenhuis, R., and Webber, R.: Iterative Restoration of Tomosynthetic Slices. International Symposium on Medical Images and Icons, Arlington, Virginia, July 24-27, 1984.

Ruttimann, U., and Webber, R.: A Simple Model Combining Quantum Noise and Anatomical Variation in Radiographs. Medical Physics, 11(1), Jan/Feb 1984, pp 59-60.

Webber, R., Ruttimann, U., and Groenhuis, R.: Computer Correction of Projective Distortions in Dental Radiographs. J. Dent. Res., 63: 1032-1035, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00065-13 DS

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Development and Evaluation of Improved Diagnostic Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

Webber, R.L.	Chief Diagnostic Sy	NIDR	DS
Ruttimann, U.E.	Senior Staff Fellow	NIDR	DS
Groenhuis, R.A.J.	Visiting Scientist	NIDR	DS

COOPERATING UNITS *(if any)*

Childrens Hospital National Medical Center
Radiation Physics Group, National Bureau of Standards

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN YEARS	PROFESSIONAL	OTHER
3.19	1.40	1.79

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)* This project is an extension of previous work directed toward the study of noninvasive methods to determine spatial and temporal relationships existing between tissues of clinical interest. The approach involves in vitro modelling of promising systems and the development of prototypes suitable for clinical evaluation.

Recent work has focused on studies directed toward development of a versatile computerized radiographic system designed to be used both fluoroscopically and off-line to produce images which can be subtracted to show small changes in tissue occuring over long intervals of time, and combined in ways permitting tomographic display of specific slices of individual teeth.

An all-electronic prototype has been fabricated which couples a specially developed intraoral x-ray source having a 10 focal spot which can be positioned electronically in a circular locus on an extended target, to a video-based fluoroscopic image-intensifier system.

Preliminary tests with a cadaver specimen show that clinically meaningful subtraction images result from selection of appropriate projections taken on two different occasions without the need for an occlusal stint to stabilize projection geometry. Other tests confirm earlier computer simulations which demonstrated the ability to reconstruct any desired tomosynthetic slice from the same eight circularly distributed radiographic projections exhibiting a total angular disparity of only eight degrees.

Other work has centered on the analysis of variations in x-ray projections produced by small changes in projection geometry. A rigorous model has been developed and tested which enables the application of statistical theory to determine the detectibility of specified lesions by ideal systems in the presence of empirically determined changes common to conventional radiographic practices. Related work also demonstrated that some of the observed variance attributable to small changes in projection geometry can be eliminated retrospectively by means of suitable affine and quadratic transformations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00211-08 DS

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Enhancement and Processing of Diagnostic Images

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name title, laboratory, and institute affiliation)*

Webber, R.L.	Chief, Diagnostic Sy	NIDR	DS
Ruttimann, U.E.	Senior Staff Fellow	NIDR	DS
Groenhuis, R.A.J.	Visiting Scientist	NIDR	DS

COOPERATING UNITS *(if any)*

Radiation Physics Group, National Bureau of Standards
United States Army Institute of Dental Research

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.68	1.40	1.28

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This project is an extentions of previous work involving the creation, development and testing of image processing techniques designed to improve diagnostic performance.

Current work has centered on methods relevant to the processes of radiologic image subtraction and tomosynthesis. Work continues in the area of automated manipulation to facilitate registration. Particular emphasis has been placed on the selection of task-specific measures of image similarity and the use of warping transformations to facilitate restoration of projective distortions.

Other related research involves the development and testing of an algorithm designed to estimate the area and volume of localized osseous lesions from dental radiographs. Preliminary data correlate well with measurements of size and volume measured directly on radiologic phantoms.

Three different approaches have been studied which reduce unwanted blur produced by tomosynthetic reconstruction from radiographic images. One involves iterative correction of a given tomosynthetic plane made possible by selective subtraction of blurred signals produced by convolution of structures in that plane and kernels determined by the exposure geometry. The other two are simple deconvolution techniques, one linear and the other nonlinear.

The iterative method was shown to converge to a set known to contain the correct solution even in the presence of uncorrelated noise. However, it also was found to be expensive computationally.

Psychophysical analysis of the simpler deconvolution techniques indicate that they can produce images which rival those processed iteratively in certain dental applications.

Considered en masse these findings are consistent with capabilities proposed for a prototype x-ray system currently being developed in cooperation with scientists at the National Bureau of Standards.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00373-02 DS

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Quantitative assessment of changes associated with myofacial pain dysfunction syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Roberts, D.	Visiting Scientist	NIDR	DS
Webber, R.L.	Chief, Diagnostic Sy	NIDR	DS
Pastakia, B.	Staff Radiologist	DR	CC

COOPERATING UNITS (if any)

Radiology Associates, Medical Imaging Section, University of Pennsylvania
Diagnostic Radiology Department, Clinical Center, NIH

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.50	1.20	0.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided) Efforts to quantify factors influencing myofacial pain dysfunction (MFPD) associated with occlusal dysfunction and temporomandibular joint disorders are well under way. Among the most intensively studied are new techniques for noninvasively assessing the existence and extent of clinically meaningful changes in the temporomandibular joint. These include the use of contiguous CT scans to produce a detailed three-dimensional replica of joint constituents and the use of nuclear-magnetic resonance (NMR) to produce images of intracapsular structures.

Data derived from CT scans of cadaver specimens show that the maximum error in predicting the measured volume of a human condyle using an existing algorithm is less than 5% irrespective of the projection geometry used to produce the scans. The data show also that the precision of the volume estimate is relatively independent of the parameters used to produce the data. Related, controlled investigations suggest that conventional methods for acquiring and displaying CT data produced from intracapsular tissues can produce artifacts due to "volume-sharing" within anisotropic volume elements which may result in significant errors in the diagnosis of a displaced meniscus.

NMR studies have demonstrated significant alterations in tissues produced by postmortem changes. This complicates the design of controlled experiments for calibrating observed patterns with known pathological conditions. In spite of this obstacle, new surface-coil technology already has produced images of intracapsular components which clearly depict relationships consistent with knowledge derived from direct dissection.

Other work centers on the use of photoplastic wafers to measure the distribution of occlusal forces as influenced by the presence of unilateral myofacial pain. Plastic deformations produced by occlusal stresses are analyzed photometrically in a way which facilitates automated analysis by computer. Preliminary data indicate that the use of drugs to mediate pain has little influence on the measured distribution of occlusal forces in MFPD patients.

PHS 6040 (Rev. 1/84)

GPO 904-917

ANNUAL REPORT OF THE NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

October 1, 1983 - September 30, 1984

The Neurobiology and Anesthesiology Branch is concerned with the study of oral-facial sensation, with particular emphasis on mechanisms of pain and the development of new methods for controlling pain in humans. The Branch is composed of three sections that utilize anatomical, physiological, behavioral, pharmacological and psychophysical techniques to study neural function as it relates to the processing of sensory signals about the threat of tissue-damaging stimulation. The Neural Mechanisms Section includes the following research activities: 1) correlative morphological, physiological and neurochemical studies of the organization of the medullary and spinal dorsal horns and the identification of putative neurotransmitters involved in sensory transmission; 2) correlative behavioral and physiological studies to determine the role of different peripheral and central neural populations in pain and temperature discrimination. The Neurocytology and Experimental Anatomy Section is concerned primarily with the study of synaptic connections in the medullary and spinal dorsal horns in normal tissue and following peripheral nerve injury. The Clinical Pain Section develops new methods for measuring and assessing experimental and clinical pain and applies these methods to the study of mechanisms of acute and chronic pain in humans as well as to the evaluation of new techniques for the treatment of acute and chronic pain.

We continue to focus our efforts on the role of the medullary and spinal dorsal horns in pain transmission. The functional significance of thalamic projection neurons in the superficial dorsal horn is being elucidated utilizing combined anatomical, physiological and cytochemical techniques. Combined behavioral, physiological and pharmacological studies examine the functional properties of trigeminothalamic projection neurons in animals trained to detect and discriminate noxious thermal stimuli. Different immunocytochemical methods are being used to determine the neurochemically-defined neural circuitry in the dorsal horn. Our animal studies provide a conceptual framework for human studies on mechanisms of acute postsurgical pain and chronic pain conditions. Human studies also focus on improved methods for assessing acute and chronic pain, the evaluation of non-steroidal anti-inflammatory agents useful in controlling postsurgical pain, and the development of new methods for the treatment of pain associated with neuropathies, myofascial pain, and cancer pain.

The pain research facility at the NIH was opened in March, 1983 under NIDR's leadership in the Clinical Center Ambulatory Care Research Facility. Within eighteen months the patient load has increased from 35 patients per week to over 100 patients per week. The Clinical Pain Section coordinates this multi-Institute collaborative program on pain research. Present studies are evaluating pain associated with diabetic neuropathies, postherpetic neuralgia, atypical facial pain and myofascial disorders of the head, neck and lower back. A number of collaborative studies with NCI have been initiated including the evaluation of bromadoline, a new analgesic; a study of intraoperative

splanchnicectomy for the relief of pancreatic carcinoma; and the development of delta-receptor opiate agonists as substitutes for morphine-like mu receptor agonists for the relief of pain in tolerant patients. Collaborative projects on children with cancer include morphine infusions and nitrous oxide analgesia for pain control as well as studies of the epidemiology of pain in children with cancer. Collaborative studies also have been initiated with NIMH, NHLBI, NICHHD and the Clinical Center.

Investigators in the Branch received considerable recognition for their achievements in the past year. Dr. Gobel received the USPHS Commendation Award for his excellent anatomical studies on pain pathways. Ms. Wirdzek received the NIH Merit Award for her exceptional talents in coordinating the research and clinical efforts of the pain research facility. Ms. Culnane, the CC nurse working with our clinical program was named the NIH Nurse of the Year for her research and pain management abilities. Dr. Dubner was elected to the Board of Directors of the American Pain Society and recently was nominated for President of the American Pain Society and Vice-President of the International Association for the Study of Pain. He also was Scientific Program Chairman of the 1983 American Pain Society meeting which was the most successful meeting of this society, both scientifically and in terms of attendance.

After 18 years of service to the NIDR during which time he became an internationally-recognized research scientist, Dr. Gobel transferred to the Division of Research Grants and was appointed Executive Secretary of a new study section. The loss of his expertise will be felt in the NAB program. We wish him great success in his new career.

Research accomplishments of the Branch are presented in more detail below.

The Neural Circuitry of the Medullary and Spinal Dorsal Horns

The lower end of the spinal trigeminal nucleus in the brain stem, called trigeminal nucleus caudalis, is directly continuous with the spinal dorsal horn and is homologous to it in terms of structure, chemistry and physiological function. For these reasons, it is more properly referred to as the medullary dorsal horn. This year we have continued our in-depth studies of the functional organization of the medullary and spinal dorsal horns and their role in pain mechanisms.

A major projection system in lamina I of the spinal dorsal horn presently is being studied. Neurons in lamina I were identified with physiological methods as having axons projecting to the brain in cats. Nearly all the neurons responded exclusively to noxious stimuli. Only about 2% of the neurons responded to innocuous and noxious stimuli (wide-dynamic-range neurons). Some of the lamina I projection neurons projected to the parabrachial area in the midbrain, an area that has not previously been considered a target for nociceptive inputs. Intracellular staining of these neurons revealed that they are morphologically diverse. They all appear to receive serotoninergic

input as determined by immunocytochemical techniques. This serotonin input originates in the brain stem and appears to play an important role in pain modulation. Of particular interest was our finding that there may be two populations of lamina I projection neurons, one that receives a high density of serotonin inputs and another that receives a low density. Another interesting characteristic of these lamina I projection neurons is their small axons. With the exception of some spinothalamic tract axons, their conduction velocities are much slower than those of other pathways travelling from the spinal cord to the brain.

The local neuron circuits in the deeper layers of the dorsal horn also has been investigated. The activation of low-threshold mechanoreceptive primary afferents with large, myelinated axons (A-beta afferents) is known to modulate pain perception. This modulation is the rationale for several pain therapies (e.g., dorsal column stimulation, transcutaneous nerve stimulation). There are several reasons to suspect that the A-beta fiber-induced modulation of pain is, at least partly, mediated by segmental (spinal) circuitry. A systematic search of the dorsal horn revealed several morphologically distinct populations of neurons that might mediate the ability of input from low-threshold mechanoreceptors to inhibit pain. In particular, a population of small interneurons in lamina III was identified. Their axons collateralized extensively in laminae III and IV and issued numerous boutons. Their responses to A-beta stimuli exactly coincided with the A-beta fiber-induced inhibition seen in projection neurons, i.e., they faithfully followed stimuli below 1 Hz, responded in a progressively more inconstant fashion to stimuli at 5-20 Hz, and failed to respond at all to stimuli exceeding 30 Hz. They were excited exclusively by A-beta afferents. Thus, there is very strong evidence that these small, lamina III interneurons are involved in the production of the A-beta induced inhibition seen in projection neurons.

The extent of coexistence of neurotransmitters is important to the understanding of the neurochemical organization of the nervous system. Although numerous studies have examined coexistence in cell bodies, identification of more than one neurotransmitter in an individual axon has had limited technical success. In the dorsal horn, the question of coexistence is significant since both the opioid peptide enkephalin and substance P may originate from multiple sources. These include descending axons from the brain stem, intrinsic dorsal horn neurons and, the case of substance P, primary afferent axons. The descending axons may also contain serotonin. To determine the location and density of coexistent axons, and the enkephalin and substance P axons which do not contain serotonin, we have employed immunocytochemical experimental paradigms. One approach uses sequential labeling of axons with two different fluorochromes. The technique can be used when antibodies produced in different species are available. It allows direct labeling of the same axon. To validate the method, coexistence of neurotransmitters in the ventral horn of the spinal cord was examined. Our observations suggest that almost all of the ventral horn substance P axons coexist with serotonin while most of the serotonin axons contain neither substance P nor enkephalin. Enkephalin axons in the ventral

horn do not appear to coexist with either substance P or serotonin. The analysis of coexistent axons and multiple neurotransmitters in the dorsal horn is now underway.

These studies are providing details on how the multitude of chemical mediators localized to the dorsal horn are organized. Knowledge of the synaptic circuitry of the dorsal horn should lead to the development of new pain control agents that alter pain without affecting non-nociceptive systems. The analysis of substance P, enkephalin and serotonin is of particular importance since all of these chemical messengers have been implicated in pain and analgesia.

Other studies have examined the effects of injury to peripheral nerves and their endings on synaptic circuitry in the medullary and spinal dorsal horns. These studies in the spinal cord and trigeminal brain stem nuclear complex show that extensive transsynaptic degeneration of second order neurons takes place after injury to the peripheral or central processes of primary neurons. It will be important to determine whether such changes in dorsal horn circuitry provide the morphological substrate for pathophysiological mechanisms associated with some chronic pain states.

Behavioral Correlates of Neural Function in the Medullary Dorsal Horn

We have extended our analysis of the neuronal properties of the medullary dorsal horn by correlating response characteristics with behavior in awake monkeys trained in sensory discrimination tasks. Our major objectives this year were 1) to determine discrimination levels to thermal stimuli in humans and monkeys utilizing newly developed behavioral tasks and to study neural responses in the medullary dorsal horn while monkeys performed these tasks; 2) to study the effects of drugs applied locally in the medullary dorsal horn on the activity of thermally-sensitive neurons and on the ability of monkeys to detect noxious and innocuous thermal stimuli; and 3) to study the role of attentional factors in sensory discrimination.

In a thermal detection task, monkeys were trained to detect small changes in temperature in the noxious range. The monkey initiated a behavioral trial by pressing an illuminated panel button. Following button press, a contact thermode positioned on the upper lip increased in temperature from a baseline of 35°C to a noxious (44-48°C) temperature. After a period ranging from 3 to 9 seconds, the probe increased an additional 0.2 to 1.0°C. The monkeys were given a fruit juice reward if the panel button were released within two seconds of the onset of the second temperature shift. Monkeys detected threshold second temperature shifts of approximately 0.1° and 0.2°C from 46°C and 45°C temperatures, respectively. Detection latencies also were inversely related to the magnitude of suprathreshold second temperature shifts and also were dependent on the first noxious temperature level. Human subjects had similar detection thresholds and latencies in this task. These findings demonstrate that monkeys and humans can make fine discriminations in the pain sensitivity range. They also indicate that detection latencies provide a measure of the perceived intensity of

small suprathreshold temperature shifts of 0.1° to 1.0°C in the noxious heat range.

Neuronal activity was recorded from trigeminothalamic and non-projecting wide-dynamic-range and nociceptive-specific neurons while monkeys performed the behavioral detection task. There was a monotonic relationship between the magnitude of discharge of neurons and second temperature levels for almost all neurons sensitive to temperature changes in the noxious heat range. Since both neuronal discharge and detection latencies were monotonically related to second temperature levels, they were directly compared. Detection latency was inversely related to the magnitude of discharge from both 45°C and 46°C first temperature shifts. Furthermore, the magnitude of neuronal discharge predicted the behavioral detection latency since the relationship was similar independent of noxious temperature levels. Intensities of first temperature shifts (45° or 46°C) and second temperature shifts (0.2-0.8°C) that produced similar peak neuronal discharge frequencies also had similar detection latencies. These findings indicate that both wide-dynamic-range and nociceptive-specific neurons provide the minimal information necessary for the detection of small temperature changes in the noxious heat range and that these specialized central nociceptive pathways are essential for such fine discriminations.

We conclude from these correlative behavioral and neural studies that nociceptive neurons in the medullary dorsal horn code stimulus intensity in the noxious heat range and that performance of this detection task is dependent on activation of this central nociceptive pathway projecting to the thalamus. The findings support the concept that pain is signalled by specialized central pathways carrying information about stimulus quality, intensity and location.

One cannot conclude from the above findings that activation of specialized nociceptive pathways always produces pain. There is evidence that these pathways are subject to considerable modulation. Recent studies provide evidence that these pain signals can be modified at the first central synapse in the spinal cord or the brain stem. What role do these descending control systems play in the processing of sensory information under natural behavioral conditions? In our behavioral studies we examined whether behavioral variables such as attention modify the monkeys' ability to detect noxious heat stimuli. In these studies the term 'attention' refers to a selective phenomenon that influences an animals' response to competing environmental stimuli. The effect of selective attention on behavioral responses to competing thermal and visual stimuli was assessed. The monkeys were trained in the thermal detection task described above. They also were trained to perform a visual detection task in which the monkeys were rewarded for releasing the response button within 2 sec of the onset of a cue light rather than a second temperature shift. Visual and thermal trials were presented randomly and the monkeys could not anticipate the relevant cue in a trial. To aid in the detection, on some thermal trials a signal was presented throughout the trial indicating that the reinforced cue was a temperature shift. Similarly, on some visual trials a different signal was presented throughout the trial indicating that the reinforced

cue was to be the cue light. The signal lights allowed the monkeys to attend to the relevant cue on that trial and we examined whether such information improved their performance of the task. Both monkeys tested in this attentional task responded with shorter mean latencies on signalled than on unsignalled thermal trials. The mean percentage of undetected trials ('no responses') across sessions was smaller on signalled than on unsignalled trials. Thus, attentional manipulations influenced both the detectability of intensity changes in the noxious heat range and the latency of correct detections. Although we do not know whether these attentional effects on noxious heat detection are reflected in changes in activity of trigeminothalamic neurons, the neural substrate of selective attention likely involves modulatory systems active in specialized nociceptive pathways.

Morphine and endogenous opioid substances activate descending pain modulatory pathways and act directly on dorsal horn nociceptive neurons. It commonly is assumed that morphine-like drugs produce clinical analgesia by altering the unpleasantness or aversiveness of pain sensations. Since dorsal horn neurons are part of specialized nociceptive pathways, these opiate drugs should also alter sensory-discriminative aspects of pain. To further elucidate the action of opiates, we have microinjected morphine into the monkey medullary dorsal horn and examined its effects on the detection of noxious heat. Monkeys were trained in the thermal detection task described previously and varying doses of morphine were injected. There was a dose-dependent effect of morphine on a monkey's ability to detect suprathreshold (approximately 90% correct detections) second temperature shifts of 0.4°C from a 46°C baseline. The effects of morphine microinjection persisted for up to 2 hrs and could be reversed by systemic administration of the opiate receptor antagonist naloxone. These behavioral changes were not related to non-specific attentional or motoric effects of morphine since the monkeys were able to simultaneously perform an innocuous cooling detection task of similar difficulty with no change in detection latencies. The results confirm our previous findings in humans that opiate drugs have a pharmacologically-specific effect on sensory-discriminative capacities in the noxious range. They also show that the site of action of these effects is at an early stage of sensory processing in the medullary dorsal horn. The findings provide additional support for a specialized role for dorsal horn neurons in coding stimulus intensity information in the noxious range.

The presence of behavioral and pharmacological modulation of dorsal horn neuronal activity provides strong evidence that these specialized nociceptive pathways are not immutable. Other findings suggest that these nociceptive pathways can be activated by behaviorally-relevant stimuli that are unrelated to pain discrimination. Some medullary dorsal horn projection and non-projection nociceptive neurons activated by noxious thermal stimuli exhibit additional responses during behavior that are independent of stimulus parameters or stimulus modality. These task-related responses sometimes occur at trial initiation and sometimes after the signal to release the response button, irrespective of whether that cue was an innocuous thermal or visual stimulus. Similar stimuli

that do not have any behavioral relevance outside of the task, do not produce these responses. Task-related responses are associated with sensory cues that are components of the sequence of behavioral events leading to reception of the reward.

These data show that the neural representation of oral-facial nociception can be influenced by environmental and behavioral factors at the earliest stage of central neural integration and that these signals are relayed to a thalamic nucleus that receives thermal sensory information. In addition, the microinjection data demonstrate local pharmacological modulation of nociception directly within the medullary dorsal horn, in the absence of generalized perceptual or motoric effects. The findings further suggest a direct role of this area in pain discrimination and its modulation and can ultimately lead to new and important methods of pain control.

The Assessment of Experimental and Acute Clinical Pain

The purpose of these human studies is 1) to develop psychophysical and behavioral models of pain perception that assess the intensity and unpleasantness of experimental and clinical pain sensation, and 2) to use these models to assess physiological and psychological mechanisms of pain and analgesia, and the efficacy of pharmacological and non-pharmacological methods of pain control.

Recently developed psychophysical methods permit us to separate subjects' ratings of the intensity of pain sensations from their ability to rate the pain properly. A previous study showed that intravenous fentanyl resulted in analgesia without a decrease in psychophysical performance, and intravenous diazepam decreased psychophysical performance without producing analgesia. Preliminary analysis of a new study in which analgesia and sedative drugs were presented orally shows that a narcotic reduced the intensity of the tooth pulp sensations and also interfered with psychophysical ability. Oral diazepam did not alter either the intensity of the tooth sensations or the ability to describe them. The results show that this model is sensitive to oral as well as intravenous narcotic agents. A main feature of the model is an internal check for scaling performance and a validity criterion that must be met before the data are accepted. Comparison to the previous study suggests that effects on scaling performance are not a fixed property of a drug class but also depend on route of administration and experienced side effects.

An additional study developed alternative forms of the Descriptor Differential Scale (DDS) for rating dimensions of the pain experience. The DDS scale presents multiple measures, thereby decreasing variability and providing a measure of internal consistency to assess scaling performance. These alternative forms were cross-validated in a new study. These forms were used in a study of pain memory (pain following third molar extractions) that examined the effect of expectation and previous ratings of experienced pain on pain recall. Memory for pain experience is usually regarded as quite poor. The study of pain memory found that the magnitude of postsurgical pain was recalled with a high

degree of accuracy one week after surgery. Recall accuracy was not influenced by expectation or by rating pain immediately after surgery. All longitudinal studies of pain and studies of pain memory have used the same pain measures repeatedly. Thus repeated measures are confounded with memory of previous responses. The development of alternative forms is a major advance because it avoids this confound.

We recently have completed a study assessing the effects of morphine, naloxone and placebo on thermal sensations mediated by A-delta and C-fiber primary afferents. An analysis of 16 subjects showed that morphine increased the latency to sensations mediated by either A-delta or C-fiber primary afferents. Latencies to auditory stimuli were unaffected, suggesting that the increased thermal latencies found after morphine administration represent an attenuation of thermal sensory intensity and not a generalized motoric deficit. Morphine also significantly reduced the ability to detect the onset of intense, centrally summated, C-fiber mediated second pain sensations, suggesting that the intensity of these sensations was reduced to near-threshold levels. Spinal pain-summation mechanisms may play an important role in chronic pain states. Temporal summation of C-fiber pain sensations is attenuated dramatically by morphine, suggesting a possible important mechanism of opioid analgesia.

Assessment and Treatment of Chronic Pain

We are continuing to evaluate the effects of narcotic analgesics and electrical brain stimulation on clinical and experimental pain in a group of chronic pain patients, some of whom received chronic brain electrode implants for pain relief. These electrodes are placed in brain pathways where they are presumed to activate descending, opiate-related, pain-suppressing systems. Sixteen patients participated in this study this year. Eight were admitted for a screening visit, ten participated in a preoperative evaluation, and three received implanted electrodes and participated in a postoperative evaluation. Results are similar to those found previously. Morphine significantly reduced the magnitude of clinical pain in the preoperative assessments and this effect was reversed by naloxone. In contrast, the analgesia produced by brain stimulation in the postoperative evaluation was no greater than that observed after sham stimulation. This analgesia was not reversed by the narcotic antagonist naloxone.

The use of deep brain stimulation to control human pain evolved from the findings in animals that electrical stimulation of periaqueductal sites activated a descending analgesic system mediated by endogenous opioid-like compounds. The human brain stimulation procedure is assumed also to activate a descending opioid system. Our previous findings that the stimulation-produced analgesia does not show an opioid time course and is not reversed by a narcotic antagonist suggest that the analgesia is not produced by an opioid mechanism. The reduced analgesia found in many patients after one year questions the efficacy and ultimate clinical utility of this procedure.

Some of our newer clinical trial studies evaluate mechanisms of pain and new treatment plans for deafferentation pain associated with cancer. Diabetic neuropathy pain and shingles pain are chronic pain syndromes associated with sensory loss, hyperalgesia and dysesthetic sensations. They are poorly treated with conventional analgesics. We are evaluating the efficacy of tricyclic antidepressant drugs in the treatment of these painful syndromes. These agents are thought to increase the availability of serotonin levels in the brain and may act by influencing descending pain-suppressing pathways. Cancer pain studies are evaluating new agents in patients tolerant to morphine. These agents act on opiate receptors not sensitive to morphine administration and thus not made tolerant to morphine. Such studies should provide alternative treatment possibilities for cancer patients with severe pain even after exposure to very high doses of morphine.

Chronic, intractable facial pain is often associated with depression, which forms the rational for antidepressive drug administration in patients with atypical facial pain. While antidepressive drugs are being utilized extensively both for the treatment of depression and chronic pain, there is conflicting evidence as to the mode of action of antidepressant drugs on pain and on depression. We have initiated a study to evaluate the efficacy of amitriptyline in controlling pain in patients with atypical facial pain, and also are studying the interrelationship between chronic pain, depression, sleep disorder and ratings of acute pain. Preliminary data indicate a strong placebo effect in almost all patients, with the superiority of the high dose of amitriptyline over placebo or low dose. It is possible, however, that pain relief is not due to the antidepressive quality of the drug.

Control of Pain and Anxiety in Ambulatory Dental Patients

These investigations are evaluating novel drugs for controlling postoperative pain in an attempt to identify agents which possess greater analgesic efficacy or less side effect liability than standard agents. A within-subject, double-blind crossover design is being employed in these investigations. Patients in need of bilateral extraction of impacted third molars serve as subjects. Flurbiprofen, a non-steroidal anti-inflammatory agent, in combination with etidocaine, a long-lasting local anesthetic, was compared to standard treatment. The combination of flurbiprofen and etidocaine resulted in less postoperative pain then standard treatment with oxycodone plus acetaminophen and lidocaine. Approximately forty percent of the patients in the sample had no postoperative pain during the seven hour observation period at the clinic following the experimental combination. A recently completed factorial study replicating the previous findings indicates that the results of the two investigational drugs are additive. Etidocaine was demonstrated to suppress peak pain intensity in comparison to lidocaine anesthesia. Flurbiprofen resulted in less overall postoperative pain than did the combination of oxycodone and acetaminophen. The combination of etidocaine and flurbiprofen resulted in less pain than either drug alone. Sixty-seven precent of the patients reported either 'none' or 'slight' pain after the combination, in comparison to 17% in the standard treatment group. These findings

indicate that a substantial propertion of patients experience little or no pain following this treatment.

A parallel investigation evaluated a new narcotic antagonist analgesic, conorphone, in comparison to codeine, a standard orally-effective narcotic analgesic. Both doses of conorphone resulted in analgesia comparable to the 60 mg dose of codeine. Conorphone, however, resulted in a significantly higher incidence of side effects than codeine. These findings suggest that conorphone may not be clinically useful as an alternative to codeine.

The objective of other studies is to evaluate the modification by drugs of the neurohumoral, psychological and physiologicl responses to acute pain and apprehension in patients undergoing a stressful surgical procedure, the removal of impacted third molars. Prototype drugs employed include placebo, anti-anxiety agents, narcotic analgesics and their antagonists. We have evaluated the pituitary release of beta-endorphin into the circulation during surgical stress. In an initial study, blood samples were collected prior to and during oral surgery. A significant increase in circulating endorphin levels was seen during surgery which was associated with subjects' intraoperative report of anxiety and discomfort. Results of the main study indicate that both fentanyl and diazepam suppress beta-endorphin levels while naloxone, an opiate antagonist, increases levels in comparison to placebo. These findings suggest a role for the pituitary release of beta-endorphin in the response to surgical stress. These investigations are clarifying the role of endogenous mechanisms of pain inhibition. Better understanding of these dynamic processes will likely result in more rationale pharmacological approaches to the management of pain.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

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Anatomical Studies of Trigeminal Sensory Nuclei and Spinal Dorsal Horn

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gobel, Stephen	Chief, NEA Section	NIDR NA
Sugimoto, Tomosada	Visiting Fellow	NIDR NA
Humphrey, Emma L.	Biol. Lab. Tech. (Elec. Mic.)	NIDR NA
Allen, Barbara V.	Biologist	NIDR NA

COOPERATING UNITS (*if any*)

Weinblatt, Anita, ATCC, Rockville, Maryland
Fromm, Gerhard, Neurology Dept., Univ. of Pittsburgh, Pittsburgh, Pennsylvania

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neurocytology and Experimental Anatomy Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.80	.75	1.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type Do not exceed the space provided.*)

This project examines the synaptic connections between primary neurons which make up trigeminal and spinal nerves on one hand and the neurons of the medullary and spinal dorsal horns on the other hand especially those in the substantia gelatinosa of Rolando. This project also considers the form, synaptic connections and function of the neurons of the dorsal horn as well as their pathological responses to peripheral nerve injuries, trigeminal and dorsal root injuries and herpes virus infections. These studies employ electron microscopy, the Golgi method, degeneration techniques and the use of intraneuronal markers such as horseradish peroxidase and neurotoxins. The goals of these studies are to delineate trigeminal and spinal pain-temperature pathways, to understand the effects of different pathologic processes on these pathways and to broaden our understanding of oral-facial sensations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-16 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Design and Computer Interfacing of Neurophysiologic Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, Frederick J.	Electronic Engineer (Instru)	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology & Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.00	1.00	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These projects involve the development of electronic and electromechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Electronic circuit design, microcomputers and assembly language programming are used in these instruments and the interfacing equipment connected to laboratory or multipurpose computer installations.

PHS 6040 (Rev 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00132-10 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

CT 006102

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Pharmacological Modification of Neuroendocrine Responses to Surgical Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Butler, Donald P.	Sr. Staff Den (Oral S)	NIDR CIPC
Hargreaves, Kenneth M.	Postdoctoral Fellow	NIDR NA
Wirdzek, Peggy R.	Nurse Specialist	NIDR NA
Schmidt, Elizabeth A.	Clinical Nurse	NIDR NA

COOPERATING UNITS *(if any)*

Goldstein, David G., NHLBI, HE

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.30	1.25	1.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The purpose of this project is 1) to objectively evaluate the efficacy and clinical toxicity of drugs given to outpatients to alleviate apprehension associated with dental procedures, 2) to study the physiological, psychological and biochemical responses to the stress of dental therapy, and 3) to evaluate the role of exogenous epinephrine administered with local anesthetic on cardiovascular performance. Special attention has been given to the non-invasive measurement of cardiac output and stroke volume by thoracic impendance cardiography. A recent study employing this methodology indicates that exogenous epinephrine administered with local anesthesia results in an increase in circulating epinephrine levels and that there is a concommitant increase in cardiac output. A parallel investigation indicated that the elevated epinephrine levels and elevated cardiac output are not attentuated by diazepam premedication. These findings suggest that exogenously administered epinephrine results in an increase in circulating levels and a resultant increase in cardiac output. A separate investigation demonstrated that the stress of surgery results in a measureable increase in plasma levels of beta-endorphin. Current investigations indicate that fentanyl and diazepam block this response while naloxone, an opiate antagonist, results in elevated beta-endorphin levels in comparison to placebo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00133-10 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

CT 0060101

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)

Assessment of Experimental and Clinical Pain

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

Gracely, Richard H.	Research Psychologist	NIDR NA
Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Maixner, William	PRAT Fellow	NIDR NA
Kwilosz, Donna M.	Psychologist	NIDR NA

COOPERATING UNITS (*if any*)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.00	.70	1.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The objectives of this project are (1) to assess psychophysical methods of experimental pain measurement, i.e., magnitude estimation, category scaling, and cross-modality matching. Pain will be experimentally induced by electrocutaneous, electric tooth pulp, and mechanical heat stimulation; (2) to assess clinical pain measures, such as pain questionnaires and sensory matching methods, in a dental setting; (3) to determine the validity of experimental pain models by comparison of experimental and clinical pain responses; and (4) to evaluate known pharmacological and non-pharmacological pain-control agents.

Results of a pain scaling task termed functional measurement show that this method is sensitive to oral and intravenous narcotics and also can assess scaling performance. Changes in performance may depend on the type of drug administered, on the route of administration, and on experienced side effects.

Alternative forms of a verbal clinical pain measure have been developed to avoid the confound between recall of pain and recall of previous pain ratings. These forms are highly reliable and have been used to assess memory of postoperative dental pain. Although pain memory is regarded as poor, pain magnitude was recalled at one week with a high degree of accuracy.

A new interactive, computer-based scaling method has been developed that can provide temporal and dose-response information in a single subject, and that avoids assumptions required by other psychophysical procedures. Initial results show that the method is sensitive to both heat applied to the skin and electrical stimulation of intact teeth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00246-07 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Pharmacological Management of Chronic Orofacial Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sharav, Yair	Visiting Scientist	NIDR NA
Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Gracely, Richard H.	Research Psychologist	NIDR NA
Max, Mitchell B.	Neurologist	NIDR NA
Singer, Elyse	Medical Staff Fellow	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.60	1.15	.45

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of the present phase is to evaluate the relationship between administration of diazepam and ibuprofen and the clinical symptoms, psychological state and neurohumoral responses of patients with Tempromandibular Pain Dysfunction Syndrome (MPD). Subjects with MPD are administered diazepam, ibuprofen, diazepam and ibuprofen or placebo for a 4-week period. Pain and mandibular function are evaluated as well as the effect of treatment on anxiety and depression. Beta-endorphin and cortisol levels are being assessed from blood samples of these patients. These data are analyzed to determine the effect of the drugs used.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00247-07 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Cytomorphology of Functionally Characterized Spinal Dorsal Horn Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Bennett, Gary J.	Senior Staff Fellow	NIDR NA
Hayashi, Haruhide	Visiting Associate	NIDR NA
Hylden, Janice	PHS Postdoc Fellow	NIDR NA
Kamogawa, Hiroyuki	Visiting Fellow	NIDR NA
Ruda, Maryann T.	Research Biologist	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
4.05	3.35	.70

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Neurons in lamina I were antidromically identified as having axons projecting to the brain (lamina I projection neurons) and then physiologically characterized with respect to their cutaneous sensitivity. Nearly all of the neurons were found to respond exclusively to noxious stimuli. Some of the neurons were determined to project to the parabrachial area, an area in the brain stem that has not been previously considered as a target of nociceptive inputs. Intracellular staining showed that lamina I projection neurons belong to diverse morphological types. Immunocytochemical techniques showed that lamina I projection neurons in general, as well as those that project to the parabrachial area, are invariably contacted by serotonergic axons that descend from the raphe nuclei. A systematic search of the dorsal horn revealed several morphologically distinct populations of neurons that might mediate the ability of input from low-threshold mechanoreceptors to inhibit pain. In particular, a population of small interneurons in lamina III was identified and found to have the electrophysiological and anatomical characteristics appropriate for interneurons producing the inhibitory postsynaptic potentials seen in projection neurons after the activation of low-threshold mechanoreceptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00276-06 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Brain Stimulation Analgesia in the Control of Chronic Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Max, Mitchell B.	Neurologist	NIDR NA
Kwilosz, Donna M.	Psychologist	NIDR NA
Wirdzek, Peggy R.	Nurse Specialist	NIDR NA

COOPERATING UNITS *(if any)*

Lees, David E., CC ANES
Young, Ronald, UCLA, Los Angeles, California
Smoller, Bruce, Psychiatrist, Bethesda, Maryland

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.95	.70	1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of the study are (1) Assess the effectiveness of chronic electrical stimulation of midbrain sites for the relief of chronic pain in humans; (2) Evaluate the efficacy and mechanisms of traditional narcotic analgesia and compare these to chronic electrical stimulation of midbrain sites; (3) Validate experimental models of pain and their potential diagnostic use in chronic pain patients; and (4) Determine and compare the impact of both traditional narcotic and chronic electrical stimulation therapies on the functional, intellectual and emotional well being of these patients. Participants in this study will be (1) chronic pain patients receiving surgically implanted stimulating electrodes for pain control; (2) chronic pain patients maintained on traditional narcotic analgesics who will not receive implanted stimulating electrodes; and (3) healthy normal volunteers. The effects of chronic brain stimulation in surgical patients will be compared to the effects of narcotics previously administered to patients and to effects of narcotic regimes in nonsurgical chronic pain patients. In addition, the effects of narcotics on perceptual and neural mechanisms of experimentally induced pain will be assessed in pain-free volunteers.

Results from 16 patients this year support previous findings from this project. Morphine significantly decreased the magnitude of low back pain and this effect was reversed by naloxone. The effect of deep brain stimulation was no greater than that observed after sham stimulation and this effect was not reversed by naloxone. The thermal pain studies showed that morphine altered pain sensations mediated by both A-delta and C-fiber primary afferents, and in addition, attenuated the central C-fiber summation observed with delivery of repetitive stimuli delivered at fast rates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00286-05 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984 CT 0060133

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Evaluation of Oral Analgesics for Ambulatory Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Wirdzek, Peggy R.	Nurse Specialist	NIDR NA
Butler, Donald P.	Sr. Staff Den (Oral S)	NIDR CIPC
Fox, Philip C.	Dental Officer	NIDR CIPC

COOPERATING UNITS *(if any)*

Culnane, Mary, CC

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.20	.25	.95

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The project consists of studies (1) to evaluate the efficacy of two novel pain control agents, flurbiprofen and etidocaine; (2) to evaluate the interaction of flurbiprofen and etidocaine when given in combination to suppress postoperative pain; (3) to evaluate the analgesic efficacy of a novel narcotic antagonist analgesic, conorphone, in comparison to a standard narcotic, codeine.
Both etidocaine and flurbiprofen resulted in greater pain suppression than standard drugs and the combination of the two resulted in greater analgesia than either drug alone. This combination possesses potential for improvement in the treatment of postoperative pain in that 67% of the sample reported little or no pain following the surgical removal of impacted molars, a procedure which normally results in moderate to severe postoperative pain. The analgesic effect of flurbiprofen was demonstrated to be partially dissociated from its other anti-inflammatory effects, suggesting that such drugs do not produce analgesia solely by suppressing inflammation. Conorphone was demonstrated to result in analgesia comparable to codeine but also resulted in significantly greater side effects suggesting that it is not a rational alternative to codeine in ambulatory patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00288-05 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropharmacological Characterization of Synaptic Circuitry in Dorsal Horn

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ruda, Maryann T.	Research Biologist	NIDR NA
Collantes, Margarita E.	Biologist	NIDR NA
Tashiro, Takashi	Visiting Fellow	NIDR NA
Humphrey, Emma L.	Biol. Lab. Tech. (Elec. Mic.)	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.63	.83	1.80

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The past year has been used in large part to develop new approaches to identify the neural circuitry of pain and analgesia in the dorsal horn. Progress has been made in two areas. First, we have developed two approaches to identify the location and density of axons which contain coexistent neurotransmitters. One approach utilizes masking of immunofluorescence by an immunoperoxidase label. A second approach uses two different immunofluorescent markers. Serotonin, enkephalin and substance P innervation of the ventral horn of the spinal cord was used as a test system. In the ventral horn almost all substance P axons were found to contain serotonin while most of the serotonin axons contained neither substance P nor enkephalin. Analysis of dorsal horn axons with coexistent neurotransmitters has begun.

A second area of development is the production of monoclonal antibodies to a subpopulation of dorsal horn neuronal elements. Monoclonal antibodies will allow subpopulations of neurons to be isolated for further analysis. These studies have recently been initiated. A cell suspension of nervous tissue, enriched with thalamic projection neurons, has been harvested and used for immunization of mice. An antibody to thalamic projection neurons will provide further information on the organization of this important system in pain transmission.

The development of the thalamic projection system in the rat has been examined as an adjunct to our experiments with monoclonal antibodies. In neonates, few thalamic projection neurons could be labeled prior to postnatal day 10. In the trigeminal system at postnatal day 10, projection neurons appear to be concentrated at the rostral pole of nucleus caudalis. These observations suggest that the trigeminothalamic and spinothalamic systems are late to mature. Noxious information in neonates may thus be carried to higher centers of the neuraxis by a separate neuronal system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00291-05 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microinjection of Morphine in the Medullary Dorsal Horn of the Behaving Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Oliveras, Jean-Louis	Visiting Fellow	NIDR NA
Maixner, William	PRAT Fellow	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Kenshalo, Daniel R.	Senior Staff Fellow	NIDR NA
Bushnell, Mary C.	Senior Staff Fellow	NIDR NA
Bates, Renee	Psychologist	NIDR NA
Thomas, David Alan	Psychologist	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.65	1.85	.80

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies the effects of morphine on the capabilities of behaving monkeys to detect temperature variations in the noxious range applied to the face. Rhesus monkeys are trained to detect small temperature increments (0.4°C, 0.6°C, 1.0°C) from a 46°C baseline. In another task, the monkeys are trained to discriminate 47°C from innocuous temperature. Additionally, the animals have to perform either a visual task where the cue is the onset of a light or an innocuous thermal cooling task where they have to detect a decrease of 0.5°C from a 38°C baseline. Morphine sulfate is directly administered in the medullary dorsal horn by microinjection technique (0.2 μl in 2 minutes). Morphine reduces in a dose-dependent manner (1 μg - 10 μg) the capabilities of the monkeys to detect the small change of 0.4°C from 46°C. This effect is reversed by the systemic administration of the specific opiate antagonist naloxone. Larger amounts of morphine sulfate (10 μg) are required to attenuate the detection of 0.6°C and 1.0°C. In addition the same dose of 10 μg is able to suppress the discrimination of 47°C. Even with large amounts (10 μg and 15 μg) morphine does not affect the capabilities to detect the onset of a light or a small 0.5°C decrement in the cooling range. These data suggest that morphine can specifically attenuate noxious thermal discrimination when directly applied to the medullary dorsal horn without altering detection and perception of innocuous thermal or visual stimuli.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00312-04 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunocytochemistry of Identified Spinal Dorsal Horn Laminae I and IIa Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dubner, Ronald	Chief, NAB	NIDR NA
Ruda, Maryann T.	Research Biologist	NIDR NA
Miletic, Vjekoslav	Postdoctoral Fellow	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.80	.60	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurons in laminae I and II of the lumbar spinal cord of the cat were characterized physiologically in terms of responsiveness to various natural stimuli (such as pinch and brush), peripheral input (C fiber vs. A-delta vs. A-beta), and effects of nucleus raphe magnus stimulation. These neurons were then intracellularly injected with horseradish peroxidase, the cats perfused, and the spinal cord sectioned and reacted with diaminobenzadine. The neurons were identified, the tissue processed immunohistochemically with antibodies to serotonin and substance P, and the cells and immunoreactive boutons then drawn by camera lucida technique with high power light microscopy. Major anatomic features, including dendritic branching pattern, dimensions of branch segments, and numbers of contacts on shafts and spines of dendritic segments, were stored in the NIDR PDP 11/70 computer. Programs were written to analyse general anatomic features of subpopulations of neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00313-04 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conjoint Measurement Analysis of Metric and Nonmetric Stimuli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NIDR NA

COOPERATING UNITS *(if any)*

Heft, Marc W., NIA, LBS

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.05	.05	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this study are to assess the contributions of category meanings and positions on category lists on the perceptual scale values for those items. Subjects rate differences between stimulus pairs consisting of an electric shock intensity and a word or category number from three distinct seven-point category lists. Perceptual scale values for both the electric shock and category items are determined by Conjoint Measurement analysis. Comparisons of the derived exponents for Stevens' power functions for electric shock show that subjects are performing the tasks similarly in three experiments, so comparison of the perceptual scales for the category items is appropriate. Results from these comparisons show that category meanings rather than positions are important in determining perceptual scale values. In addition, subjects estimate the magnitude of sensations evoked by electrical stimulation. Power functions also describe the relation between the numerical judgments and currents. In almost all instances the derived exponents for electric shock determined in the difference estimation experiment were less than those determined in the single-stimulus experiments, consistent with results from other perceptual continua.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00329-03 NA

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Discrimination of Thermal Stimuli Applied to the Face in Monkey and Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Maixner, William	PRAT Fellow	NIDR NA
Kenshalo, Daniel R.	Senior Staff Fellow	NIDR NA
Bushnell, Mary C.	Senior Staff Fellow	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Duncan, Gary H.	Clinical Dental Assoc	NIDR NA
Bates, Renee	Psychologist	NIDR NA
Wheeling, Howard S.	PRAT Fellow	NIDR NA
Thomas, David Alan	Psychologist	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
2.30	1.50	.80

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project correlates behavioral responses with neural responses of thalamic projection and non-projection neurons in the medullary dorsal horn (trigeminal nucleus caudalis) produced by noxious thermal stimuli in the behaving monkey. Medullary dorsal horn neurons encode thermal discriminative information used by the monkey to perform a thermal detection task. Many medullary dorsal horn neurons encode thermal intensity in a manner which allows the detection of small changes in noxious temperatures. Certain subpopulations of medullary dorsal horn neurons appear to encode thermal intensity information better at near threshold detection levels than other thermally responsive medullary dorsal horn neurons. In addition, some thermally sensitive neurons also respond to other stimuli used by the monkey for the successful completion of the task. This task-related activity occurs in characteristic patterns of excitation and/or inhibition and some neurons which exhibit such activity project to the thalamus. The neurons examined in this study appear to be important in encoding noxious sensory discriminative information. The task-related responses exhibited by some of these neurons may modulate sensory activity and thereby influence the perception of and response to oral-facial pain. In addition, this project studies the ability of rhesus monkeys and humans to detect small changes in innocuous warm pulses and noxious heat pulses applied to the face and the influence of attention on thermal discriminative capacity. A signal correctly indicating the location or modality of a subsequent thermal change improves detection performance while an incorrect signal worsens performance. Attentional manipulations have a greater influence on the ability to detect changes in noxious heat as compared to innocuous heat. Consequently, attentional factors may influence the perception of and response to oro-facial pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00366-02 NA

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Analgesic Mechanisms in Patients with Chronic Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Max, Mitchell B.	Neurologist	NIDR NA
Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Gracely, Richard H.	Research Psychologist	NIDR NA
Wirdzek, Peggy R.	Nurse Specialist	NIDR NA

COOPERATING UNITS *(if any)*
Chang, Alfred, NCI
Culnane, Mary, CC
Wall, Russell, CC

LAB/BRANCH
Neurobiology & Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.13	.75	.38

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The purpose of this work is to evaluate drug therapy in chronic pain syndromes that are considered resistant to known approaches: syndromes caused by injury to nerve, such as diabetic neuropathy and herpes zoster, and syndromes caused by advanced cancer in which tolerance to the analgesic effects of narcotics has developed.

A 12-week study of patients with painful diabetic neuropathy is underway. Amitriptyline is compared to active placebo under double-blind conditions. Patients record levels of pain and mood 5 times a day. The effect of drug on perception of heat by nociceptive nerve fibers is evaluated during clinic visits. The data will be analyzed to learn if amitriptyline is effective in this condition, and if the effects on pain and mood are independent. Analysis of data from the first 11 patients show an analgesic effect; 29 of the full cohort of 30 patients have been accrued.

Patients with post-herpetic neuralgia enter a similar chronic drug study, comparing amitriptyline to lorazepam to placebo, as well as a single-dose analgesic study, comparing codeine, ibuprofen, clonidine and placebo; all of these drugs may work by interacting with analgesic systems in the injured nerve or adjacent spinal cord. Twenty-five of a planned 40 patients are enrolled.

In advanced cancer, conventional opiate drugs may lose their effectiveness because of the development of tolerance at the mu opiate receptor. Studies are planned using stable analogues of endogenous enkephalins, which produce analgesia by action at the delta-receptor; these agents retain effectiveness in animals made tolerant to mu-receptors. The relative potency of intrathecal DADL-enkephalin and morphine will be compared in patients with varying degrees of tolerance to morphine, and the relative potency of intramuscular metkephamid to morphine will be compared in morphine-naive and tolerant patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00377-01 NA

PERIOD COVERED

January 1, 1984 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Responses of Primate SI Cortical Neurons to Noxious Temperature Increments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kenshalo, Daniel R.	Senior Staff Fellow	NIDR NA
Dubner, Ronald	Chief, NAB	NIDR NA
Wheeling, Howard S.	PRAT Fellow	NIDR NA
Bates, Renee	Psychologist	NIDR NA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.23	.73	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study examines the ability of monkeys to detect small temperature changes in the noxious range applied to the wrist. Once the animal is trained to detect small temperatures, the response of somatosensory cortical neurons will be correlated with the monkey's ability to detect small increments in temperature in the noxious range. In addition, the monkey's ability to detect small thermal increments on the wrist are compared with previous results obtained from the face. These data provide a direct comparison between the relative sensitivities of the face and of the wrist to thermal stimulation in the noxious range.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00378-01 NA

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Amitriptyline in the Relief of Orofacial Pain, Depression and Sleep Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sharav, Yair	Visiting Scientist	NIDR NA
Dionne, Raymond A.	Senior Staff Fellow	NIDR NA
Gracely, Richard H.	Research Psychologist	NIDR NA
Schmidt, Elizabeth A.	Clinical Nurse	NIDR NA
Max, Mitchell B.	Neurologist	NIDR NA
Singer, Elyse	Medical Staff Fellow	NIDR NA

COOPERATING UNITS *(if any)*
Smoller, Bruce, Psychiatrist, Bethesda, Maryland

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: .95	PROFESSIONAL: .85	OTHER .10

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of lower and higher dosages of amitriptyline (30 and 150 mg/day) are studied in patients with Atypical Facial Pain. The following are being evaluated: chronic pain, depression, anxiety, rating of acute pain induced by heat application to the skin, quality of sleep and sleep disorders and beta-endorphin blood levels. A double-blind incomplete cross-over design of drug administration is used and each subject is administered in two 4-week periods two of the following: placebo, high or low dose of amitriptyline.

COLLABORATIVE RESEARCH

-PROJECT NUMBER......3N01DE02424-06 PI ADDRESS
PARKER, NORMA

AWARD AMOUNT.........$0 ILLINOIS

INSTITUTION..........AMERICAN DENTAL ASSN HEALTH FDN
TITLE Role of dentists and pediatricians in caries prevention

Conduct a national survey through a written questionnaire administered to a stratified probability sample of practicing dentists and pediatricians to assess: the extent of knowledge of current caries prevention methods among dental practitioners and pediatricians; the acceptance and use of these procedures among practitioners; the extent to which practicing dentists and pediatricians advise patients regarding caries prevention; and the characteristics of dentists and pediatricians who are aware of and receptive to preventive philosophies.

-PROJECT NUMBER......3N01DE02426-05

PI ADDRESS
MICHALEK, SUZANNE M

AWARD AMOUNT.........$15,998

ALABAMA

INSTITUTION..........UNIVERSITY OF ALABAMA IN BIRMINGHAM
TITLE   Immunize rodents with nonfreund-type anticaries vaccines

Determine the efficacy and suitability of incorporating minimally toxic non-Freund-type adjuvants into anti-caries vaccines. These vaccines will be used to immunize rodents. Antibody responses and immunoglobulin levels will be measured in the serum and saliva of these animals. Following viable cariogenic bacterial challenge, the dental caries that develop will be scored. Histopathological examinations of tissues at the injection sites will also be made. From the many categories of adjuvants currently available, it is anticipated that trials with new formulations will give rise to well-tolerated, minimally toxic preparations which will elicit good titers of protective antibodies.

-PROJECT NUMBER......3N01DE12430-08 PI ADDRESS

DALGARD, DANIEL W

AWARD AMOUNT.........$244,869 VIRGINIA

INSTITUTION..........HAZLETON LABORATORIES CORPORATION

TITLE Investigation of anticaries vaccine in primates

This procurement is for veterinary care, quarantine procedures, technical support care, and housing for research animals which are presently part of an investigation in anti-caries immunization with sub-human primates. The Contractor will house approximately 70 primates (Macaca fascicularis) in accordance with the highest standards of animal husbandry.

-PROJECT NUMBER......3N01DE12431-04 PI ADDRESS

MELLBERG, JAMES R

AWARD AMOUNT.........$0 NEW YORK

INSTITUTION..........STATE UNIVERSITY NEW YORK STONY BROOK

TITLE Clinical trail of a combined MFP-NaF dentifrice

A three year longitudinal clinical trial of MFP-NaF dentifrice formulated at different fluoride concentrations. The purposes of this procurement are: to evaluate the anticaries efficacy of a combined MFP-NaF dentifrice with a total fluoride concentration of 1000 ppm; and to determine whether the efficacy of this formulation can be improved by raising the total fluoride concentration to 2500 ppm. A standard MFP dentifrice with a fluoride concentration of 1000 ppm will be used as a positive control. Both experimental formulations will contain NaF and MFP at concentrations equimolar with respect to fluoride.

-PROJECT NUMBER......2N01DE12433-04 PI ADDRESS

BANDT, CARL L

AWARD AMOUNT.........$302,213 MINNESOTA

INSTITUTION..........UNIVERSITY OF MINNESOTA OF MNPLS-ST PAUL

TITLE Phase contrast evaluation of subgingival plaque

Determine: reliability of phase contrast microscopic evaluation of subgingival plaque as a correlate of periodontal inflammatory status; utility of phase contrast microscopic evaluation of subgingival plaque as a diagnostic and prognostic tool; utility of videotape recording of phase contrast microscopic observation of subgingival plaque as a means of patient motivation; and relative efficacy of a combined regimen of flossing, irrigation, and regularly patient-applied hypertonic salts in oxidative antiseptic, compared with a conventional home treatment regimen of flossing and commercial dentifrice in the control of the subgingival flora and periodontal inflammation.

-PROJECT NUMBER......3N01DE12434-06

PI ADDRESS
CURZON, MARTIN E

AWARD AMOUNT.........$23,055

NEW YORK

INSTITUTION..........EASTMAN DENTAL CENTER
TITLE Identify cariogenic elements of food

A research study of the cariogenic potential of common snack foods. The cariogenicity of foods will be assessed by the method in which rats are fed their essential nutrition by gastric intubation, and only the test foods--fed through a programmed feeder--are allowed to come in contact with the animals' teeth. The study will also include salivary function measurements and microbial implantation measurements.

-PROJECT NUMBER......3N01DE22438-05 PI ADDRESS

BURT, BRIAN A

AWARD AMOUNT.........$0 MICHIGAN

INSTITUTION..........UNIVERSITY OF MICHIGAN AT ANN ARBOR
TITLE Relationship of dietary intake to caries incidence (human)

The University of Michigan will conduct a four-year longitudinal study in which associations between various dietary intake patterns and the incidence of dental caries are investigated. Multiple short-term dietary intake measurements will be collected from approximately 800 school children, ages 11-12, who reside in a community where the drinking watercontains less than 0.3 ppm fluoride. All clinical examinations will be performed by NIDR dentists and the data will be provided to the University of Michigan. The statistical analyses of the dietary and clinical data will be performed by the Contractor.

-PROJECT NUMBER......3N01DE22439-04 PI ADDRESS
DOGON, I LEON

AWARD AMOUNT.........$224,933 MASSACHUSETTS

INSTITUTION..........FORSYTH DENTAL CENTER
TITLE Cost of combined sealant and self-applied fluoride program .

A study of the cost, feasibility, and effectiveness of training and utilizing preventive dentistry aids for the deliveryof caries preventive agents and techniques in a three-year, school-based program. The objective of the proposed study is to determine the maximum level of prevention of caries currently achievable in a school-age population at a cost level acceptable to the average U.S. consumer.

-PROJECT NUMBER......3N01DE32400-04
PI ADDRESS
RIPA, LOUIS W

AWARD AMOUNT.........$82,073
NEW YORK

INSTITUTION..........STATE UNIVERSITY NEW YORK STONY BROOK
TITLE   NaF mouthrinsing for caries prevention

This project is a three year longitudinal "double blind" clinical trial to study the effect of mouthrinsing with a daily0.05 percent neutral sodium fluoride rinse upon the development of root and coronal caries in adults. The study population will consist of approximately 1200 people, 18-65 years of age, who reside in a community where the drinking water contains less than 0.3 ppm fluoride and where the population is relatively less stable (less tha 15% annual attrition rate). The individual subjects must have a high caries susceptibility and at least 50% of the natural teeth, excluding third molars.

-PROJECT NUMBER......3N01DE32441-03

PI ADDRESS
LEVERETT, DENNIS H

AWARD AMOUNT.........$103,691

NEW YORK

INSTITUTION..........EASTMAN DENTAL CENTER
TITLE Effect of prenatal fluoride in preventing dental caries (human)

This is a five-year longitudinal "double blind" clinical trial to determine the effect of prenatal fluoride supplements in preventing the development of dental caries in the primary dentition. The study population will consist of approximately 1,200 women who are receiving pre-natal care, are in the first three months of pregnancy, and reside in areas with no more than 0.3 ppm fluoride in the drinking water. During pregnancy, a randomly selected group of women will receive daily fluoride supplements (1 mg F) while a control group will receive a placebo supplement. Upon completion of the pregnancy, a regime of daily fluoride supplements to the offspring will be prescribed. Dental examinations of all children will be conducted at approximately the time of their third and fifth birthdays.

* NO SUMMARY AVAILABLE FOR THIS PROJECT

-PROJECT NUMBER......1N01DE32443-00 PI ADDRESS

EKLUND, STEPHEN A

AWARD AMOUNT.........$0 MICHIGAN

INSTITUTION..........UNIVERSITY OF MICHIGAN AT ANN ARBOR

TITLE Effect of severe dental fluorosis on the oral health

A cross-sectional, epidemiologic survey to determine the longterm, oral health effects of severe dental fluorosis in two study populations of adults who are continuous residents of communities in New Mexico with documented 60-year histories of natural fluorides. One group shall have consumed water with fluorides at an optimal F concentration, and the other group, a water supply with at least four times the optimal F concentration. The oral health status of the study populations wil be assessed for fluorosis, coronal caries, root caries, periodontal disease, tooth wear, functional masticatory disorders, and dental plaque and calculus.

* NO SUMMARY AVAILABLE FOR THIS PROJECT

-PROJECT NUMBER......3N01DE32444-01 PI ADDRESS

DAVIDSON, WILLIAM M

AWARD AMOUNT.........$6,406 MARYLAND

INSTITUTION..........UNIVERSITY OF MARYLAND AT BALTIMORE

TITLE Pharmacology of intraoral fluoride-releasing device

A clinical pharmacology study to evaluate two intraoral fluoride-releasing devices. The study will determine fluoride-release performance, durability, and patient acceptance and tolerance of two devices designed to release 0.05 mg and 0.10 mg of fluoride/day for six months in 40 children, aged 11 through 18. Samples of saliva, dental plaque, urine, and serum will be collected throughout the study to monitor fluoride levels, and oral tissues will be monitored for signs of irritation. The study population will consist of 40 noninstutionalized prospective patients of the University of Maryland Dental School who reside in an optimally fluoridated community.

* NO SUMMARY AVAILABLE FOR THIS PROJECT

-PROJECT NUMBER......1N01DE42551-00

PI ADDRESS
MICHALEK, SUZANNE M

AWARD AMOUNT.........$170,530

ALABAMA

INSTITUTION..........UNIVERSITY OF ALABAMA IN BIRMINGHAM
TITLE Develop antigens and adjuvants for carries immunity

Develop a safe, effective, and otherwise attractive vaccine against dental caries for human use. Establish in a rat model the most effective caries-prevention combination of antigen, adjuvant, and dosing regimen using the gastric route of immunization. Crude S. mutans preparations and then purified S. mutans antigens will be tested with selected particulate adjuvants for effects on plasma and salivary immunoglobulins, oral S. mutans levels, and caries.

-PROJECT NUMBER......1N01DE42552-00

PI ADDRESS
CAUFIELD, PAGE W

AWARD AMOUNT.........$242,100

ALABAMA

INSTITUTION..........UNIVERSITY OF ALABAMA IN BIRMINGHAM
TITLE PREVENT MATERNAL TRANSMISSION OF STREPTOCOCCUS MUTANS

A total of 140 timed-pregnant rhesus monkeys were treated during early gestation with either BAPN (4 animals), Dilantin (7), Thalidomide or E-122 (4), Cytoxan (116), Bendectin (5), or pyridoxine (4). Although the number of treated animals was too small for definitive conclusion, developmental anomalies were not detected in offspring of animals treated with BPN, Dilantin, Bendectin or pyridoxine. In contrast, Cytoxan treatment during early gestation resulted in a high incidence of malformations in the offspring. These malformations varied, depending on the time of treatment, but consisted primarily of either cleft palate and/or cleft lip or craniofacial dysmorphia with either meningoencephalocele or persistent anterior fontanel. Nursery-raised infants that were prenatally exposed to Cytoxan showed a high mortality rate, and at autopsy, often showed severe thymic hypoplasia, suggestive of an induced immuno-deficiency. The frequency and reproducibility of the Cytoxan-induced anomalies suggest that infants treated in this manner could be developed as animal models for these relatively common human disorders.

* NO SUMMARY AVAILABLE FOR THIS PROJECT

-PROJECT NUMBER......3N01DE52484-10

PI ADDRESS
YANKELL, SAMUEL L

AWARD AMOUNT.........$24,522

PENNSYLVANIA

INSTITUTION..........UNIVERSITY OF PENNSYLVANIA
TITLE Antiplaques/food additives/other agents--Anticaries

Clinical testing of potential antiplaque and antimicrobial agents. Agents shall be applied both singly and in combination in vehicles such as mouthrinses and gels in various regimens and durations. Test groups shall not consist of more than 50 subjects. All testing shell be short term, i.e. 1-16 weeks. CORE MEASUREMENTS - plaques extent; dry weight, ph; microflora, gingivitis. ELECTIVE MEASUREMENTS - thickness; biochemistry; cervicular flow photography, p0.

-PROJECT NUMBER......3N01DE92422-11
PI ADDRESS
ELLISON, SOLON A

AWARD AMOUNT.........$0
NEW YORK

INSTITUTION..........COLUMBIA UNIVERSITY
TITLE Dental plaque and saliva from gastric intubated patients

Investigate a minimum of 25 gastric-intubated patients to perform detailed analysis of the microbiological, immunological, and biochemical constituents in dental plaque and saliva. Control subjects will be an equivalent number of age-matched volunteers who are staff, students, or patients at the Columbia-Presbyterian Medical Center. Samples will be taken from plaque which will be allowed to develop for four days after prophylaxis. Saliva samples will be collected after the initial cleaning prior to plaque accumulation.

PROJECT NUMBER......Y01DE20001-00

PI ADDRESS
MOFFA, JOSEPH
BETHESDA
MARYLAND

AWARD AMOUNT.........$77,037

INSTITUTION..........INDIAN HEALTH SERVICE
TITLE Clinical evaluation of restorative materials

Partial support for Interagency Agreement number Y01 DE20003 with the Department of the Army for research which involves the clinical evaluation of base-metal alloys and the nickel sensitivity problem, composite resins, amalgam alloys, and improve the complete technology of these tasks. In vitro research will also be done on ceramic and dental casting alloys.

PROJECT NUMBER......Y01DE40001-00

PI ADDRESS
MOFFA, JOSEPH
FORT DETRICK
MARYLAND

AWARD AMOUNT.........$103,522

INSTITUTION..........U.S. ARMY MEDICAL RESEARCH AND DEV. COMMAND
TITLE Clinical and laboratory evaluations of restorative materials

A. Evaluate the long-term clinical performances of amalgam, composite resin, and base-metal alloy restorations.

B. Evaluate the clinical performance of a test hydrophobic composite resin based upon a polyfluorinated oligomeric multifunctional methacrylate (PFMA) formulation as compared to a control BIS-GMA formulation for the restoration of anterior teeth.

C. Evaluate the clinical performance of a new castable ceramic material for use in fixed prosthodontics. Particular emphasis shall be placed upon the incidence of fracture, fit, and esthetic qualities of the restoration, and permanence of the superficial stain and glaze. Assessment will be based upon the placement and evaluation of 40 anterior and 40 posterior full veneer porcelain jacket crowns.

PROJECT NUMBER......Y01DE10007-03

PI ADDRESS
MOTZ, J W
WASHINGTON
D C

AWARD AMOUNT.........$0

INSTITUTION..........U.S. NATIONAL BUREAU OF STANDARDS
TITLE   Dental radiographic system with selective geometry

The investigators of this project will attempt to design and build an experimental dental radiographic source-detector system having electronically controlled exposure geometry. The detector of the system shall yield a time varying electronic signal which when interfaced to a cathode ray display system shold be capable of producing radiographic data amenable to reproducible geometric registration from one examination to the next to facilitate sequential analysis of common dental pathologies, i.e., periodontal disease, caries, etc.

This system will be specifically designed to interface with an image processing computer to be used for experimental research by the Diagnostic Methodology Section of the NIDR.

PROJECT NUMBER......Y01DE20003-03

AWARD AMOUNT.........$0

PI ADDRESS
MOFFA, JOSEPH P
SAN FRANCISCO
CALIFORNIA

INSTITUTION..........U.S. LETTERMAN ARMY INSTITUTE OF RESEARCH
TITLE Clinical and laboratory evaluations of dental restorative materials

The Contractor will perform the following studies:

1. Evaluate Base-Metal Alloys and Ceramic Materials.
a. Determine the relationship between nickel and chromium sensitivity and the presence of nickel and chromium fixed prosthodontic alloys.
b. Develop a ceramic firing regimen which will minimize residual stresses and simplify the technique.

2. Evaluate Composite Resins.
a. Evaluate the clinical performance of composite resins utilized for the restoration of posterior teeth.
b. Quantify the occlusal wear of composite resins.

3. Evaluate Amalgam Alloys.
a. Evaluate the long-term assessment of the clinical performance of previously placed amalgam alloy formulations.
b. Determine whether polishing an amalgam alloy contributes towards its longevity.

4. Improve Computer Technology in Clinical Research.
Improve the clinical computer capabilities.

PROJECT NUMBER......Y01DE30001-01

PI ADDRESS
TESK, JOHN A
WASHINGTON
D C

AWARD AMOUNT.........$496,889

INSTITUTION..........U.S. NATIONAL BUREAU OF STANDARDS
TITLE Interactions of oral structures and restorative materials

The objective of the research program is generlly to provide the science and engineering base which will lead to improved dental materials and techniques for the practice of preventive and restorative dentistry.

The NIDR-NBS supported program is divided into three major areas of research:

I. Dental composites, resins, cements and adhesion: synthesis and evaluation.
II. Wear and degradation resistance and related physical properties of dental composite restorative materials.
III. Dental alloys and ceramics.

The past year has seen the attainment of a number of significant milestones. The development of high strength, eugenol-free cements has been accomplished. Hydrophobic composite resin formulations have been developed that have lower water sorption than conventional BIS-GMA (Bis Phenol Glycidal Methacrylate). Studies on the effects of techniques for evaluating the aging of dental resins showed that exposure to a Xenon lamp produced greater changes than exposure to an RS lamp which in turn produced greater change than exposure under water to the Xenon lamp. New, more stable, initiator systems such as cumene hydroperoxide and color stable polythiols such as PETMP were formulated together to produce more color and storage stabel resin systems. Flexible backbone dimer and trimer acids were shown to produce hydrophobic materials having high durability. Wear and degradation studies showed further the importance of environment-affected wear. A simple equation was found to apply for describing the castability of each of six nickel based dental casting alloys. A unique four-point bend test specimen is used for porcelain-fused-to-metal strength determinations.

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